

(19)



(11)

EP 3 568 024 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:

25.11.2020 Bulletin 2020/48

(51) Int Cl.:

A23C 9/12 (2006.01)

C12R 1/225 (2006.01)

C12N 9/38 (2006.01)

A23C 9/123 (2006.01)

C12R 1/46 (2006.01)

(21) Application number: **18704863.2**

(86) International application number:

PCT/EP2018/050708

(22) Date of filing: **12.01.2018**

(87) International publication number:

WO 2018/130630 (19.07.2018 Gazette 2018/29)

(54) **PROCESS FOR PRODUCING A FERMENTED MILK PRODUCT**

VERFAHREN ZUR HERSTELLUNG EINES FERMENTIERTEN MILCHPRODUKTS

PROCÉDÉ DE PRODUCTION D'UN PRODUIT LAITIÉ FERMENTÉ

(84) Designated Contracting States:

**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR**

(30) Priority: **13.01.2017 EP 17151378**

(43) Date of publication of application:

20.11.2019 Bulletin 2019/47

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(56) References cited:

WO-A1-2007/021204

WO-A1-2015/193449

US-A1- 2009 088 391

WO-A1-2009/071539

WO-A1-2015/193459

US-A1- 2015 086 675

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Description

FIELD OF THE INVENTION

5 **[0001]** The present invention relates to a process for producing a fermented milk product.

BACKGROUND OF THE INVENTION

10 **[0002]** WO 2009/071539 discloses a lactase originating from *Bifidobacterium bifidum*, which is capable of very efficient hydrolysis in milk, and which is active over a broad pH range, including low pH, e.g. a pH below 5. The lactase may be used in processes for producing milk and fermented milk products, such as cheese, yogurt, butter, butter milk, sour cream etc., for reducing the content of lactose.

15 **[0003]** WO 2013/160413 discloses a method of producing a fermented milk product using a combination of glucose-negative lactic acid bacteria strains and a conventional lactase with an object of reducing the content of lactose in the fermented milk product while increasing the content of glucose.

20 **[0004]** EP-A1 -2 957 180 in one embodiment discloses a method of producing a fermented milk product using a combination of a starter cultures and a conventional lactase with an object of reducing content of lactose and the level of post-acidification in the fermented milk product. EP-A1-2 957 180 in a second embodiment discloses a method of producing a fermented milk product using lactose-deficient lactic acid bacteria.

SUMMARY OF THE INVENTION

[0005] The object of the present invention is to provide an improved process for producing a fermented milk product.

25 **[0006]** The object of the present invention is obtained by a process for producing a fermented milk product comprising the steps of

- 1) adding a starter culture comprising at least one lactic acid bacteria strain to a milk base,
- 2) fermenting the milk for a period of time until a target pH is reached,
- 3) wherein the starter culture comprises at least one lactose-deficient strain, which is capable of metabolizing a non-lactose carbohydrate, and
- 30 4) adding a low pH stable lactase to the process either at the start, during or at the end of the fermentation step, wherein the low pH stable lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

35 **[0007]** Lactose-deficient lactic acid bacteria typically grow on a non-lactose carbohydrate source, such as sucrose, galactose and glucose, added to the milk in an amount measured so as stop the fermentation process and the growth of the lactic acid bacteria by depletion of the added carbohydrate source. Hereby, the post-acidification during subsequent storage is lowered significantly or even fully prevented. A low pH stable lactase will be active during the full course of a fermentation process and hence allows conversion of most or all of the lactose present in milk to glucose and galactose. Hereby, it is possible to produce a fermented milk product with a reduced content of lactose or a lactose-free product. Also, it is possible to produce a fermented milk product with an increased natural sweetness, as glucose and galactose has a much higher sweetness than lactose.

40 **[0008]** The present invention is based on the recognition that by using a combination of a low pH stable lactase and lactose-deficient lactic acid bacteria, it is possible to obtain a fermented milk product, which at the same time has reduced lactose content, an increased sweetness and reduced post-acidification. Furthermore, it has surprisingly been found that the said combination results in a fermented milk product having an increased texture as compared to using lactose-deficient lactic acid bacteria and no lactase and as compared to using a low pH stable lactase and lactose-positive lactic acid bacteria.

45 **[0009]** Furthermore, when the lactase is added before the fermentation step the process of the invention has provided a possibility of reducing or even eliminating the amount of non-lactose carbohydrate to be added to the milk. Thus, the glucose and galactose formed by the enzymatic action of lactase will be available for the growth of the lactose-deficient lactic acid bacteria.

[0010] Finally, when the lactase is added after the fermentation step, it is possible to take full advantage of the ability of the lactose-deficient lactic acid bacteria to avoid post-acidification.

55 **[0011]** Worldwide, a significant numbers of consumers are intolerant or sensitive to lactose. Therefore, there is presently a high demand for dairy products, including fermented milk products, with a reduced content of lactose or which is substantially free of lactose. The present invention has provided a new approach for producing such product in a simple and cost-efficient manner.

DETAILED DISCLOSURE OF THE INVENTION

Lactase

[0012] The lactase of the fermented milk product of the invention may be any lactase, which retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

[0013] In relation to the present invention the activity in LAU of the lactase is measured as specified in the "Definitions" section below.

[0014] In a preferred embodiment of the invention, the lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 10 %, preferably at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum pH of the lactase.

[0015] In a preferred embodiment of the invention, the lactase retains its activity at a pH of 4.0 and a temperature of 37 °C at a level of at least 5 %, preferably at least 10 %, more preferably at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum pH of the lactase.

[0016] In a preferred embodiment of the invention, the lactase retains its activity at a pH of 3.0 and a temperature of 37 °C at a level of at least 5 %, preferably at least 10 %, more preferably at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum pH of the lactase.

[0017] In connection with the present invention the optimum pH of the lactase is determined by measuring the lactase activity at pH using the method indicated in the "Definitions" section below and determining the pH with optimum activity. In a preferred embodiment of the invention, the lactase retains its activity at a temperature of 10 °C and a pH of 6.0 at a level of at least 10 % as compared to its activity at the optimum temperature of the lactase. Preferably, the lactase retains its activity at a temperature of 10 °C and a pH of 6.0 at a level of at least 20 %, more preferably at least 30 %, more preferably at least 40 %, more preferably at least 50 %, more preferably at least 60 %, more preferably at least 70 %, and most preferably at least 80 %, as compared to its activity at the optimum temperature of the lactase.

[0018] In connection with the present invention the optimum temperature of the lactase is determined by measuring the lactase activity at different temperatures using the method indicated in the "Definitions" section below and determining the temperature with optimum activity.

[0019] In a preferred embodiment, the lactase to be used in the product of the present invention has a lactase activity at 37°C and pH 5 which is at least 55%, such as at least 60%, at least 65%, at least 70% or at least 75%, of its lactase activity at 37°C and pH 6.

[0020] In another preferred embodiment, the lactase to be used in the product of the present invention has a lactase activity at 37°C and pH 4.5 which is at least 10%, such as at least 20%, at least 30%, at least 35% or at least 40%, of its lactase activity at 37°C and pH 6.

[0021] In another preferred embodiment, the lactase to be used in the product of the present invention has a pH optimum of the lactase activity at 37°C which is above pH 5.5.

[0022] In another preferred embodiment, the lactase to be used in the product of the present invention has a lactase activity at a temperature of 52°C and a pH of 6.5 which is at least 50%, such as at least 55%, at least 60%, at least 65%, at least 70%, at least 75% or at least 80%, of its lactase activity at a temperature of 38°C and a pH of 6.5.

[0023] In a preferred embodiment of the present invention, Km of the lactase at 5°C is below 25 mM, such as below 20 mM, below 15 mM or below 10 mM. In another preferred embodiment, Km of the lactase at 37°C is below 25 mM, such as below 20 mM or below 15 mM. The skilled person will know how to determine Km for the lactase activity at a specific temperature. Km may be determined by the method described in WO2009/071539.

[0024] In another preferred embodiment, the enzyme when hydrolyzing the lactose in the milk product has a ratio of lactase to transgalactosylase activity of more than 1:1, such as more than 2:1 or more than 3:1. In another preferred embodiment, the enzyme treatment is performed under conditions where the lactase activity of the enzyme is higher than the transgalactosylase activity, such as at least two times higher or at least three times higher.

[0025] The ratio of lactase to transgalactosylase activity in the milk product may, e.g., be determined by HPLC analysis. In another preferred embodiment, the enzyme treatment is performed under conditions where at least 50% (w/w%) of the hydrolyzed lactose is converted into free galactose. In another preferred embodiment, the enzyme treatment is performed under conditions where the hydrolyzed lactose is converted into equal amounts of free glucose and free galactose.

[0026] A lactase in the context of the present invention is a glycoside hydrolase having the ability to hydrolyze the disaccharide lactose into constituent galactose and glucose monomers. The group of lactases, to which the lactase of the invention belongs, comprises but is not limited to enzymes assigned to subclass EC 3.2.1.108. Enzymes assigned to other subclasses, such as, e.g., EC 3.2.1.23, may also be lactases in the context of the present invention. A lactase

in the context of the invention may have other activities than the lactose hydrolyzing activity, such as for example a transgalactosylating activity. In the context of the invention, the lactose hydrolyzing activity of the lactase may be referred to as its lactase activity or its beta-galactosidase activity.

[0027] Enzymes having lactase activity to be used in a method of the present invention may be of animal, of plant or of microbial origin. Preferred lactases are obtained from microbial sources, in particular from a filamentous fungus or yeast, or from a bacterium.

[0028] The enzyme may, e.g., be derived from a strain of *Agaricus*, e.g. *A. bisporus*; *Ascovaginospora*; *Aspergillus*, e.g. *A. niger*, *A. awamori*, *A. foetidus*, *A. japonicus*, *A. oryzae*; *Candida*; *Chaetomium*; *Chaetotomastia*; *Dictyostelium*, e.g. *D. discoideum*; *Kluveromyces*, e.g. *K. fragilis*, *K. lactis*; *Mucor*, e.g. *M. javanicus*, *M. mucedo*, *M. subtilissimus*; *Neurospora*, e.g. *N. crassa*; *Rhizomucor*, e.g. *R. pusillus*; *Rhizopus*, e.g. *R. arrhizus*, *R. japonicus*, *R. stolonifer*; *Sclerotinia*, e.g. *S. libertiana*; *Torula*; *Torulopsis*; *Trichophyton*, e.g. *T. rubrum*; *Whetzelinia*, e.g. *W. sclerotiorum*; *Bacillus*, e.g. *B. coagulans*, *B. circulans*, *B. megaterium*, *B. novalis*, *B. subtilis*, *B. pumilus*, *B. stearothermophilus*, *B. thuringiensis*; *Bifidobacterium*, e.g. *B. longum*, *B. bifidum*, *B. animalis*; *Chryseobacterium*; *Citrobacter*, e.g. *C. freundii*; *Clostridium*, e.g. *C. perfringens*; *Diplodia*, e.g. *D. gossypina*; *Enterobacter*, e.g. *E. aerogenes*, *E. cloacae* *Edwardsiella*, *E. tarda*; *Erwinia*, e.g. *E. herbicola*; *Escherichia*, e.g. *E. coli*; *Klebsiella*, e.g. *K. pneumoniae*; *Miriococcum*; *Myrothesium*; *Mucor*; *Neurospora*, e.g. *N. crassa*; *Proteus*, e.g. *P. vulgaris*; *Providencia*, e.g. *P. stuartii*; *Pycnoporus*, e.g. *Pycnoporus cinnabarinus*, *Pycnoporus sanguineus*; *Ruminococcus*, e.g. *R. torques*; *Salmonella*, e.g. *S. typhimurium*; *Serratia*, e.g. *S. liquefaciens*, *S. marcescens*; *Shigella*, e.g. *S. flexneri*; *Streptomyces*, e.g. *S. antibioticus*, *S. castaneoglobisporus*, *S. violeceoruber*; *Trametes*; *Trichoderma*, e.g. *T. reesei*, *T. viride*; *Yersinia*, e.g. *Y. enterocolitica*.

[0029] In a preferred embodiment, the lactase originates from a bacterium, e.g. from the family Bifidobacteriaceae, such as from the genus *Bifidobacterium*, such as from a strain of *B. bifidum*, *B. animalis* or *B. longum*. In a more preferred embodiment, the lactase originates from *Bifidobacterium bifidum*.

[0030] In a preferred embodiment, an enzyme having lactase activity to be used in the product of the present invention comprises an amino acid sequence which is at least 50% identical to a sequence selected from the group consisting of amino acids 28-1931 of SEQ ID NO: 1, amino acids 28-1331 of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and lactase active fragments thereof. In a more preferred embodiment, the enzyme comprises an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to a sequence selected from the group consisting of amino acids 28-1931 of SEQ ID NO: 1, amino acids 28-1331 of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and lactase active fragments thereof.

[0031] A preferred enzyme is a lactase having a sequence which is at least 50%, such as at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to amino acids 28-1931 of SEQ ID NO: 1 or to a lactase active fragment thereof. Such lactase active fragment of SEQ ID NO: 1 may be any fragment of SEQ ID NO: 1 having lactase activity. A lactase active fragment of SEQ ID NO: 1 may be, e.g., amino acids 28-979, amino acids 28-1170, amino acids 28-1323, amino acids 28-1331, or amino acids 28-1600 of SEQ ID NO: 1.

[0032] In a preferred embodiment, an enzyme having lactase activity to be used in the product of the present invention comprises an amino acid sequence which is at least 50% identical to amino acids 28-1331 of SEQ ID NO: 2. In a more preferred embodiment, the enzyme comprises an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to amino acids 28-1331 of SEQ ID NO: 2.

[0033] In another embodiment, an enzyme having lactase activity to be used in product of the present invention has an amino acid sequence which is at least 50% identical to SEQ ID NO: 3. Preferably, the enzyme has an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to SEQ ID NO: 3.

[0034] In another embodiment, an enzyme having lactase activity to be used in the product of the present invention has an amino acid sequence which is at least 50% identical to SEQ ID NO: 4. Preferably, the enzyme has an amino acid sequence which is at least 60%, such as at least 70%, at least 80%, at least 90%, at least 95% or at least 98% identical to SEQ ID NO: 4.

[0035] For purposes of the present invention, the degree of identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch (1970) J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al. (2000) Trends in Genetics 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labelled "longest identity" (obtained using the -no brief option) is used as the percent identity and is calculated as follows:

$$\frac{(\text{Identical Residues} \times 100)}{(\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})}$$

[0036] A particular commercial lactase suitable for use in the present invention is Lactase F "Amano" 100SD available from Amano Enzyme Europe.

[0037] Lactases to be used in a method of the present invention may be extracellular. They may have a signal sequence at their N-terminus, which is cleaved off during secretion.

[0038] Lactases to be used in a method of the present invention may be derived from any of the sources mentioned herein. The term "derived" means in this context that the enzyme may have been isolated from an organism where it is present natively, i.e. the identity of the amino acid sequence of the enzyme are identical to a native enzyme. The term "derived" also means that the enzymes may have been produced recombinantly in a host organism, the recombinantly produced enzyme having either an identity identical to a native enzyme or having a modified amino acid sequence, e.g. having one or more amino acids which are deleted, inserted and/or substituted, i.e. a recombinantly produced enzyme which is a mutant and/or a fragment of a native amino acid sequence. Within the meaning of a native enzyme are included natural variants. Furthermore, the term "derived" includes enzymes produced synthetically by, e.g., peptide synthesis. The term "derived" also encompasses enzymes which have been modified e.g. by glycosylation, phosphorylation etc., whether *in vivo* or *in vitro*. With respect to recombinantly produced enzyme the term "derived from" refers to the identity of the enzyme and not the identity of the host organism in which it is produced recombinantly.

[0039] The lactase may be obtained from a microorganism by use of any suitable technique. For instance, a lactase enzyme preparation may be obtained by fermentation of a suitable microorganism and subsequent isolation of a lactase preparation from the resulting fermented broth or microorganism by methods known in the art. The lactase may also be obtained by use of recombinant DNA techniques. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector comprising a DNA sequence encoding the lactase in question and the DNA sequence being operationally linked with an appropriate expression signal such that it is capable of expressing the lactase in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may also be incorporated into the genome of the host cell. The DNA sequence may be of genomic, cDNA or synthetic origin or any combinations of these, and may be isolated or synthesized in accordance with methods known in the art.

[0040] Lactases to be used in a method of the present invention may be purified. The term "purified" as used herein covers lactase enzyme protein essentially free from insoluble components from the production organism. The term "purified" also covers lactase enzyme protein essentially free from insoluble components from the native organism from which it is obtained. Preferably, it is also separated from some of the soluble components of the organism and culture medium from which it is derived. More preferably, it is separated by one or more of the unit operations: filtration, precipitation, or chromatography.

[0041] Accordingly, the enzyme having lactase activity may be purified, viz. only minor amounts of other proteins being present. The expression "other proteins" relate in particular to other enzymes. The term "purified" as used herein also refers to removal of other components, particularly other proteins and most particularly other enzymes present in the cell of origin of the lactase. The lactase may be "substantially pure", i.e. free from other components from the organism in which it is produced, i.e., e.g., a host organism for recombinantly produced lactase. Preferably, the lactase is at least 40% (w/w) pure enzyme protein preparation, more preferably at least 50%, 60%, 70%, 80% or even at least 90% pure.

[0042] The term enzyme having lactase activity includes whatever auxiliary compounds that may be necessary for the enzyme's catalytic activity, such as, e.g., an appropriate acceptor or cofactor, which may or may not be naturally present in the reaction system.

[0043] The enzyme may be in any form suited for the use in question, such as, e.g., in the form of a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a protected enzyme.

Lactose-deficient lactic acid bacteria

[0044] The terms "deficiency in lactose metabolism" and "lactose deficient" are used in the context of the present invention to characterize LAB which either partially or completely lost the ability to use lactose as a source for cell growth or maintaining cell viability. Respective LAB are capable of metabolizing one or several carbohydrates selected from sucrose, galactose and/or glucose or another fermentable carbohydrate. Since these carbohydrates are not naturally present in milk in sufficient amounts to support fermentation by lactose deficient mutants, it is necessary to add these carbohydrates to the milk. Lactose deficient and partially deficient LAB can be characterized as white colonies on a medium containing lactose and X-Gal.

[0045] In a particular embodiment of the invention, the lactose-deficient strain is capable of metabolizing a non-lactose carbohydrate selected from the group consisting of sucrose, galactose and glucose, preferably sucrose. In a particular embodiment of the invention, the lactose-deficient strain is capable of metabolizing galactose.

[0046] In a particular embodiment of the invention, the lactose-deficient strain is selected from the group consisting of lactose-deficient *Streptococcus thermophilus* and lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus*.

[0047] In a particular embodiment of the invention, the lactose-deficient strain is selected from the group consisting of:

(a) a *Streptococcus thermophilus* strain, which strain is:

- (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhofenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28952;
- (ii) or a strain derived from DSM 28952, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal;

(b) a *Streptococcus thermophilus* strain, which strain is:

- (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhofenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28953;
- (ii) or a strain derived from DSM 28953, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal;

(c) a *Lactobacillus delbrueckii* ssp. *bulgaricus* strain, which strain is:

- (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhofenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28910;
- (ii) or a strain derived from DSM 28910, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal.

[0048] In a particular embodiment of the invention, the starter culture contains both at least one lactose-deficient *Streptococcus thermophilus* and at least one lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus*.

[0049] In a particular embodiment of the invention, the starter culture contains both at least one *Streptococcus thermophilus* and at least one *Lactobacillus delbrueckii* subsp. *bulgaricus*, and wherein all *Streptococcus thermophilus* and all *Lactobacillus delbrueckii* subsp. *bulgaricus* strains are lactose-deficient.

Steps of the process of the invention

[0050] In a particular embodiment of the invention, the fermentation step is terminated by a method selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, 2) cooling treatment and 3) depletion of the non-lactose carbohydrate.

[0051] In a particular embodiment of the invention, non-lactose carbohydrate is added to the milk base at the start of the fermentation step.

[0052] Preferably, the non-lactose carbohydrate is added to the milk base in an amount measured so as to become depleted and hence result in stopping the growth of lactic acid bacteria and in stopping the fermentation. Preferably, the non-lactose carbohydrate is added to the milk base in an amount measured so as to become depleted and hence result in stopping the growth of lactic acid bacteria and in stopping the fermentation at a selected target pH value.

[0053] The amount of non-lactose carbohydrate to be added to the milk base depends on a number of parameters, including the lactic acid bacteria strains used in the starter culture, the composition of the milk base, the fermentation temperature and the desired target pH. Also, the amount of non-lactose carbohydrate to be added to the milk base may depend on the type and amount of lactase used in the process. The amount of non-lactose carbohydrate to be added to the milk base can be determined by experimentation, and it is well within the skills of a skilled person to carry out such experimentation.

[0054] In a particular embodiment of the invention, the added non-lactose carbohydrate is selected from the group consisting of sucrose, galactose and glucose, preferably sucrose.

[0055] In a first aspect of the invention, the low pH stable lactase is added to the milk base at the start of the fermentation step. In this aspect the added lactase will convert the lactose of the milk base to glucose and galactose, which will be available for metabolism for the starter culture in addition to the added non-lactose carbohydrate. In this case, it will not be possible to stop the fermentation by depletion of the added non-lactose carbohydrate. Thus, in a particular embodiment of the first aspect of the invention, the fermentation step is terminated by a method selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, and 2) cooling treatment.

[0056] In a particular embodiment of the first aspect of the invention no non-lactose carbohydrate is added to the fermentation step and at least one lactose-deficient lactic acid strain of the starter culture is capable of metabolizing a carbohydrate selected from the group consisting of glucose and galactose. In this embodiment the lactose-deficient lactic acid strain of the starter culture grows solely on the glucose and/or galactose formed by the enzymatic action of the low pH lactase. In this embodiment of the invention, the fermentation is stopped at a target pH value by a method

selected from the group consisting of 1) acidification of the fermented milk rendering at least one strain of the starter culture unable to grow, 2) cooling treatment and 3) depletion of the glucose and/or galactose formed by the low pH stable lactase.

[0057] In a second aspect of the invention, the low pH stable lactase is added to the fermented milk at the end of the fermentation. In this case, the lactase-containing fermented milk product is preferably stored at a temperature of at least 2 °C for at least 1 day. In a particular embodiment of the process of the invention, the lactase-containing fermented milk product is stored for at least two days, preferably at least 3 days, more preferably at least 4 days, more preferably at least 5 days, more preferably at least 6 days, and most preferably at least 7 days.

[0058] In a particular embodiment of the second aspect of the invention, the fermentation step is terminated by depletion of the non-lactose carbohydrate.

[0059] In a particular embodiment of the invention the target pH is between 3.2 and 4.8, more preferably between 3.6 and 4.6, more preferably between 3.8 and 4.5 and most preferably between 4.0 and 4.4.

[0060] In a particular embodiment of the invention the fermentation temperature is between 35 °C and 45 °C, preferably between 37 °C and 43 °C, and more preferably between 40 °C and 43 °C.

[0061] In another particular embodiment of the invention the fermentation is between 15 °C and 35 °C, preferably between 25 °C and 35 °C, and more preferably between 30 °C and 33 °C.

[0062] In a particular embodiment of the invention, the fermented milk product is packaged at a temperature between 15 and 45 °C.

[0063] In a particular embodiment of the invention, the pH value of the fermented milk product is maintained within a range of 0.3 pH units, preferably within a range of 0.2 pH units and most preferably within a range of 0.1 pH units, when stored after termination of the fermentation at the temperature used for fermentation over a period of 20 hours.

[0064] In a particular embodiment of the invention, the amount of added non-lactose carbohydrate is from 1 mg/g to 30 mg/g, preferably from 2 mg/g to 20 mg/g, and more preferably from 3 mg/g to 10 mg/g milk base.

[0065] In a particular embodiment of the invention, the amount of added non-lactose carbohydrate is from 0.1 % to 10 %, preferably from 0.2 % to 8 %, preferably from 0.3 % to 2 %, preferably from 0.4 % to 1.5 %, and more preferably from 0.5 % to 1.2 %, wherein % is (w/w) based on milk base.

[0066] The starter culture may have the strain composition of any conventional starter culture of lactic acid bacteria, including single strain culture and culture blends, used for producing a specific type of fermented milk product. Other useful bacteria, which may be added to the product in addition to the starter culture, include the probiotic bacteria *Bifidobacterium spp.*

[0067] In a particular embodiment of the invention, the fermented milk product after fermentation is subjected to a heat treatment so as to reduce the level of bacteria of the starter culture to no more than 1×10^2 CFU per g fermented milk product. In this case a particular embodiment is characterized in that the lactase is added after the heat treatment. In this case the lactase-containing fermented milk product is stored at a temperature of at least 2 °C for at least 1 day.

In a particular embodiment of the process of the invention, the lactase-containing fermented milk product is stored for at least two days, preferably at least 3 days, more preferably at least 4 days, more preferably at least 5 days, more preferably at least 6 days, and most preferably at least 7 days.

[0068] The heat treatment so as to reduce the level of bacteria of the starter culture to no more than 1.0×10^2 CFU per g fermented milk is preferably carried out by subjecting the starter culture fermented milk product to a temperature of between 50 °C and 110 °C, preferably between 50 °C and 100 °C, preferably between 50 °C and 90 °C, preferably between 60 °C and 85 °C, more preferably between 65 °C and 82 °C, and most preferably between 70 °C and 80 °C. The heat treatment is preferably carried out for a period of between 5 seconds and 180 seconds, preferably between 5 seconds and 120 seconds, more preferably between 5 seconds and 90 seconds, more preferably between 5 seconds and 60 seconds, more preferably between 8 seconds and 50 seconds and most preferably between 10 and 40 seconds.

Preferably, the level of bacteria of the starter culture is reduced to no more than 1.0×10^1 CFU per g fermented milk, more preferably 0 CFU per g. Fermented milk products subjected to a heat treatment so as to reduce the level of bacteria to no more than 1×10^2 CFU per g are suitable for use storage at ambient temperature, such as storage at a temperature of at least 5 °C, preferably at least 10 °C, more preferably at least 15 °C, and most preferably at least 20 °C.

[0069] The low pH stable lactase is added in a suitable amount to achieve the desired degree of lactose hydrolysis under the chosen reaction conditions. In a particular embodiment of the invention, lactase is added in an amount of between 100 and 20000 LAU per liter milk base, preferably between 100 and 10000 LAU per liter milk base, preferably between 100 and 5000 LAU per liter milk base, preferably less than 3000, such as less than 1500, less than 1000, less than 750 or less than 500, LAU per liter milk base.

[0070] In a preferred embodiment, the lactase is added at a concentration of between 5 and 400 LAU per g lactose in the milk base, preferably between 5 and 200 LAU per g lactose in the milk base, preferably between 5 and 100 LAU per g lactose in the milk base, preferably less than 50, such as less than 40, less than 30, less than 20 or less than 10, LAU per g lactose in the milk base.

[0071] In a preferred embodiment of the invention, the lactase is added to the milk base in an amount of between 2.0

mg/ml and 50 mg/ml, preferably between 5 mg/ml and 48 mg/ml, more preferably between 10 mg/ml and 46 mg/ml, and most preferably between 20 mg/ml and 45 mg/ml.

[0072] In a preferred embodiment of the invention, the residual level of lactose at the end of fermentation is less than 40 mg/ml, preferably less than 35 mg/ml, more preferably less than 30 mg/ml, more preferably less than 25 mg/ml, more preferably less than 20 mg/ml, more preferably less than 15 mg/ml, more preferably less than 10 mg/ml, more preferably less than 5 mg/ml, more preferably less than 3 mg/ml, and most preferably less than 1.5 mg/ml.

[0073] In a preferred embodiment of the invention, the milk base at the start of the fermentation step has a content of lactose of between 30.0 mg/ml and 70 mg/ml, preferably between 35 mg/ml and 65 mg/ml, more preferably between 40 mg/ml and 60 mg/ml, and most preferably between 50 mg/ml and 60 mg/ml.

[0074] In a preferred embodiment of the invention, wherein the low pH-stable lactase is added to the process at the end of the fermentation step, the fermented milk product, to which the lactase is to be added, has a viscosity, which allows easy distribution of the lactase in the fermented milk product, e.g. by mixing.

[0075] In a preferred embodiment of the process of the invention, the lactase to be added to the process is provided in a sterile formulation. In another preferred embodiment of the process of the invention the lactase is added to the process under aseptic conditions, e.g. by sterile filtration of a solution of the lactase.

Fermented milk product

[0076] The present disclosure further relates to a fermented milk product produced by the process of the invention.

[0077] In a particular aspect the fermented milk product produced by the process of the invention comprises the starter culture of step 3) of the said process and the low pH stable lactase added in step 4) of the said process.

[0078] In a particular aspect the fermented milk product is a product, which may be produced using a lactic acid bacteria strain selected from the group consisting of lactose-deficient *Streptococcus thermophilus* and lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus*.

[0079] In a particular aspect the fermented milk product is selected from the group consisting of yogurt, cream cheese, sour milk, sour cream, buttermilk, fermented whey, cultured milk, Smetana, Kefir, drinking yogurt, and Yakult. Preferably, the yogurt is selected from the group consisting of set yogurt, stirred yogurt and drinking yogurt.

[0080] In a preferred aspect the fermented milk product contains a further food product selected from the group consisting of fruit beverage, fermented cereal products, chemically acidified cereal products, soy milk products and any mixture thereof.

[0081] The fermented milk product typically contains protein in a level of between 2.0 % by weight to 3.5 % by weight. The fermented milk product may also be a low protein product with a protein level of between 1.0 % by weight and 2.0 % by weight. Alternatively, the fermented milk product may be a high protein product with a protein level of above 3.5 % by weight.

Use

[0082] The present invention further relates to the use in a process for producing a fermented milk product comprising the steps of

- 1) adding a starter culture comprising at least one lactic acid bacteria strain to a milk base, and
- 2) fermenting the milk for a period of time until a target pH is reached, of
- 3) the starter culture comprising at least one lactose-deficient strain, which is capable of metabolizing a non-lactose carbohydrate, and
- 4) a low pH stable lactase added to the process either at the start, during or at the end of the fermentation step, wherein the low pH stable lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

Definitions

[0083] In connection with the present invention the following definitions apply:

"LAU" means "Lactose Units" and 1 lactase unit (1 LAU) is the amount of enzyme which releases 1 micromole glucose per minute in M-buffer at pH 6.5 and 37°C with a lactose concentration of 4.75% w/v. M-buffer is prepared by dissolving 3.98 g C₆H₅Na₃O₇·2H₂O, 8.31 g citric acid, 0.9 g K₂SO₄, 2.6 g K₂HPO₄, 7.35 g KH₂PO₄, 5.45 g KOH, 4.15 g MgCl₂·6H₂O, 3.75 g CaCl₂·2H₂O and 1.4 g NaHCO₃ in 4 liter water, adding 12.5 ml 4N NaOH, adjusting to pH 6.5 using HCl, and adding water up to a total volume of 5 liter.

[0084] The activity in LAU of a specific lactase may be determined by direct measurement of glucose released from lactose under the conditions described above. The skilled person will know how to determine such activity. Alternatively,

the activity may be determined by using the lactase activity assay described in Example 1 of the present application. Here, the activity is obtained by comparing to a standard curve run with a lactase of known activity, and the activity of the unknown sample calculated from this. The lactase of known activity may, e.g., be Lactozym obtained from Novozymes A/S, Denmark.

[0085] The expression "heat treatment" means any treatment using any temperature, for any period of time and by any means or equipment, which inactivates at least a portion of the bacteria of the starter culture. In this connection the term "inactivate" means any stop, reduction or inhibition of growth of the bacteria, e.g. cell lysing.

[0086] The expression "lactic acid bacteria" ("LAB") designates a gram-positive, microaerophilic or anaerobic bacteria, which ferment sugars with the production of acids including lactic acid as the predominantly produced acid, acetic acid and propionic acid. The industrially most useful lactic acid bacteria are found within the order "Lactobacillales" which includes *Lactococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pseudoleuconostoc* spp., *Pediococcus* spp., *Brevibacterium* spp., *Enterococcus* spp. and *Propionibacterium* spp. These are frequently used as food cultures alone or in combination with other lactic acid bacteria.

[0087] Lactic acid bacteria, including bacteria of the species *Lactobacillus* sp. and *Lactococcus* sp., are normally supplied to the dairy industry either as frozen or freeze-dried cultures for bulk starter propagation or as so-called "Direct Vat Set" (DVS) cultures, intended for direct inoculation into a fermentation vessel or vat for the production of a dairy product, such as a fermented milk product or a cheese. Such lactic acid bacterial cultures are in general referred to as "starter cultures" or "starters". Typically, a starter culture for yogurt comprises *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, and in most countries a yogurt is by legislation defined as a fermented milk product produced using a starter culture comprising the two said strains.

[0088] The term "milk" is to be understood as the lacteal secretion obtained by milking of any mammal, such as cows, sheep, goats, buffaloes or camels. In a preferred embodiment, the milk is cow's milk. The term milk also includes protein/fat solutions made of plant materials, e.g. soy milk.

[0089] The term "milk base" may be any raw and/or processed milk material that can be subjected to fermentation according to the method of the invention. Thus, useful milk bases include, but are not limited to, solutions/-suspensions of any milk or milk like products comprising protein, such as whole or low fat milk, skim milk, buttermilk, reconstituted milk powder, condensed milk, dried milk, whey, whey permeate, lactose, mother liquid from crystallization of lactose, whey protein concentrate, or cream. Obviously, the milk base may originate from any mammal, e.g. being substantially pure mammalian milk, or reconstituted milk powder.

[0090] Prior to fermentation, the milk base may be homogenized and pasteurized according to methods known in the art.

[0091] "Homogenizing" as used herein means intensive mixing to obtain a soluble suspension or emulsion. If homogenization is performed prior to fermentation, it may be performed so as to break up the milk fat into smaller sizes so that it no longer separates from the milk. This may be accomplished by forcing the milk at high pressure through small orifices.

[0092] "Pasteurizing" as used herein means treatment of the milk base to reduce or eliminate the presence of live organisms, such as microorganisms. Preferably, pasteurization is attained by maintaining a specified temperature for a specified period of time. The specified temperature is usually attained by heating. The temperature and duration may be selected in order to kill or inactivate certain bacteria, such as harmful bacteria. A rapid cooling step may follow.

[0093] "Fermentation" in the methods of the present invention means the conversion of carbohydrates into alcohols or acids through the action of a microorganism. Preferably, fermentation in the methods of the invention comprises conversion of lactose to lactic acid.

[0094] Fermentation processes to be used in production of dairy products are well known and the person of skill in the art will know how to select suitable process conditions, such as temperature, oxygen, amount and characteristics of microorganism(s) and process time. Obviously, fermentation conditions are selected so as to support the achievement of the present invention, i.e. to obtain a dairy product in solid (such as a cheese) or liquid form (such as a fermented milk product).

[0095] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising", "having", "including" and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0096] The expression "fermented milk product" means a food or feed product wherein the preparation of the food or feed product involves fermentation of a milk base with a lactic acid bacteria. "Fermented milk product" as used herein

includes but is not limited to products such as thermophilic fermented milk products, e.g. yoghurt, mesophilic fermented milk products, e.g. sour cream and buttermilk, as well as fermented whey.

[0097] The term "thermophile" herein refers to microorganisms that thrive best at temperatures above 35°C. The industrially most useful thermophilic bacteria include *Streptococcus* spp. and *Lactobacillus* spp. The term "thermophilic fermentation" herein refers to fermentation at a temperature above about 35°C, such as between about 35°C to about 45°C. The term "thermophilic fermented milk product" refers to fermented milk products prepared by thermophilic fermentation of a thermophilic starter culture and include such fermented milk products as set-yoghurt, stirred-yoghurt and drinking yoghurt, e.g. Yakult.

[0098] The term "mesophile" herein refers to microorganisms that thrive best at moderate temperatures (15°C-35°C). The industrially most useful mesophilic bacteria include *Lactococcus* spp. and *Leuconostoc* spp. The term "mesophilic fermentation" herein refers to fermentation at a temperature between about 22°C and about 35°C. The term "mesophilic fermented milk product" refers to fermented milk products prepared by mesophilic fermentation of a mesophilic starter culture and include such fermented milk products as buttermilk, sour milk, cultured milk, smetana, sour cream, Kefir and fresh cheese, such as quark, tvarog and cream cheese.

[0099] In connection with the present invention, "shear stress" may be measured by the following method: Seven days after production, the fermented milk product was brought to 13°C and manually stirred gently by means of a spoon (5 times) until homogeneity of the sample. The rheological properties of the sample were assessed on a rheometer (Anton Paar Physica Rheometer with ASC, Automatic Sample Changer, Anton Paar® GmbH, Austria) by using a bob-cup. The rheometer was set to a constant temperature of 13 °C during the time of measurement. Settings were as follows:

Holding time (to rebuild to somewhat original structure)

5 minutes without any physical stress (oscillation or rotation) applied to the sample. Oscillation step (to measure the elastic and viscous modulus, G' and G'' , respectively, therefore calculating the complex modulus G^*)

Constant strain = 0.3 %, frequency (f) = [0.5..8] Hz

6 measuring points over 60 s (one every 10 s)

Rotation step (to measure shear stress at 300 1/s)

Two steps were designed:

1) Shear rate = [0.3-300] 1/s and 2) Shear rate = [275-0.3] 1/s.

Each step contained 21 measuring points over 210 s (on every 10 s).

The shear stress at 300 1/s was chosen for further analysis, as this correlates to mouth thickness when swallowing a fermented milk product.

[0100] In connection with the present invention, "gel firmness" may be measured by the following method:

Gel firmness is measured by a back extrusion test with a texture analyzer (TA.XT Plus, Stable Micro System, Surrey, UK) supplied with a 35mm parallel plate. The travel distance is set to 15 mm, and the travel speed to 2 mm/s. The test is performed after 7 days from production. The fermented milk product was brought to 13°C and manually stirred gently, and measured in a 250 g plastic container. The maximal force (N or g) obtained by force versus distance curves is used as "gel firmness" parameter, the positive area (N* mm) as degree of deformation, the maximal negative force (N) as ropiness.

[0101] The term "low pH stable lactase" herein refers to a lactase, which retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

[0102] The term "activity at the optimum pH" means the lactase activity at the pH, where the lactase has its optimum activity.

[0103] The term "non-lactose carbohydrate" means any carbohydrate, which is not lactose, and which a lactose-deficient lactic acid bacterium used in the process of the invention is capable of metabolizing.

[0104] The expression "at the start of the fermentation step" means shortly before, at the same time as or shortly after addition of the starter culture to the milk base. Here, the term "shortly" means less than 30 minutes".

[0105] The expression "during the fermentation step" means at any time during the fermentation after the start and before the end of the fermentation.

[0106] The expression "at the end of the fermentation step" means shortly before, at the same time as or shortly after the target pH is reached. Here, the term "shortly" means less than 30 minutes".

[0107] The term "target pH" means the pH at which the fermentation step ends. Depending on various parameters of the process, the fermentation step is terminated by a method selected from the group consisting of 1) acidification of

the fermented milk rendering at least one strain of the starter culture unable to grow, 2) cooling treatment and 3) depletion of the non-lactose carbohydrate.

DEPOSITS AND EXPERT SOLUTION

[0108] The Applicant requests that a sample of the deposited microorganism should be made available only to an expert approved by the Applicant.

[0109] *Streptococcus thermophilus* strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28952.

[0110] *Streptococcus thermophilus* strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28953.

[0111] *Lactobacillus delbrueckii* ssp. *bulgaricus* strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28910;

The deposits were made according to the Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

EXAMPLES

Example 1

Lactase activity-assay in Eppendorf tubes at 37°C, pH 6.5

Principle:

[0112] Lactase hydrolyses lactose into glucose and galactose. Glucose is measured after a modified version of the common glucose oxidase / peroxidase assay (Werner, W. et al. (1970) Z. analyt. Chem. 252: 224.).

[0113] LAU is defined as the amount of enzyme liberating 1 micromole of glucose per min at 37°C, pH 6.5 in M-buffer (M-buffer is defined in the description of the present patent application). Alternatively, the activity in LAU for a specific lactase may be determined by the method described here. The value obtained is compared to a standard curve run with a lactase of known activity, and the activity of the unknown sample calculated from this. The lactase of known activity may, e.g., be Lactozym obtained from Novozymes A/S, Denmark.

Solutions:

[0114] Assay buffer: 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 0.01% Triton X100, pH 6.5

[0115] GOD-Perid solution: 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP (Horse Radish Peroxidase), 0.65 g/l ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)).

Substrate:

[0116] 160 mM lactose, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, pH 6.5.

Standard:

[0117] Lactozym (available from Novozymes A/S, Denmark) with a known activity in LAU/g is used as standard, diluted in assay buffer in the range from 0.09 - 0.7 LAU/g.

Samples:

[0118] Enzyme samples are diluted appropriately in assay buffer.

Procedure:

[0119]

1. 375 μ l substrate is incubated 5 minutes at 37°C.
2. 25 μ l enzyme diluted in assay buffer is added.
3. The reaction is stopped after 30 minutes by adding 60 μ l 1 M NaOH
4. 20 μ l is transferred to a 96 well microtiter plate and 200 μ l GOD-Perid solution is added. After 30 minutes at room temperature, the absorbance is measured at 420 nm.

Example 2

[0120] 100 ml 15 or 30%(w/w) whey permeate containing primarily lactose and ions was made by mixing 15 or 30 g spray-dried whey permeate powder (Variolac, Aria) in 85 or 70 ml ionic water respectively. The solution was poured in a flask containing a magnetic stirring bar and placed in a water bath at 37°C. After 15 min, enzyme was added. Enzymes tested were Lactozym, a commercially available lactase from Novozymes A/S, Denmark, having an activity of 3060 LAU/g, and an experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 and an activity of 295 LAU/g.

[0121] Dosages were 4225 LAU/l milk of Lactozym and 2025 LAU/l milk of the *Bifidobacterium* lactase. Milk samples were taken at regular intervals up till 5.5 hrs. and the enzyme was inactivated by heating to 99°C for 10 min in a thermomixer. Samples were diluted appropriately and filtered through a 0.20 μ m filter.

[0122] Lactose hydrolysis was measured using a Dionex BioLC equipped with a Dionex PA1 column and a Pulsed Amperometric Detector (PAD). Peaks were identified and quantified by comparing with known standards of lactose, glucose and galactose.

[0123] Results are given below.

Table 1: Lactose, glucose and galactose in 15% DS whey permeate after treatment with Lactozym or *Bifidobacterium* lactase.

	Lactozym			<i>Bifidobacterium</i> lactase		
Time min	Lactose mM	Glucose mM	Galactose mM	Lactose mM	Glucose mM	Galactose mM
0	499	1	2	499	1	2
30	312	135	106	410	61	63
60	211	224	155	349	119	122
120	110	295	221	220	199	202
180	66	324	249	149	281	290
240	50	346	279	84	336	348
330	37	372	312	31	350	368

Table 2: Lactose, glucose and galactose in 30% DS whey permeate after treatment with Lactozym or *Bifidobacterium* lactase.

	Lactozym			<i>Bifidobacterium</i> lactase		
Time min	Lactose mM	Glucose mM	Galactose mM	Lactose mM	Glucose mM	Galactose mM
0	848	1	4	848	1	4
30	824	109	75	819	43	45
60	615	253	150	788	86	83
120	420	370	242	651	159	158
180	291	459	300	625	232	230
240	246	559	373	501	283	273
330	154	544	367	391	333	324
1440	54	649	545	20	727	739

[0124] Also when tested at higher lactose concentrations as in 15% or 30% whey permeate no or very little galactooligosaccharides are produced. Again, the produced galactose and glucose levels are equal and match the amount of lactose hydrolyzed. For comparison, Lactozym produces less galactose than glucose, clearly showing that galactooligosaccharides have been produced.

Example 3

[0125] pH profile (at 37°C) and temperature profile (at pH 6.5) of experimental lactase from *Bifidobacterium bifidum* using lactose as substrate.

Principle:

[0126] Lactase hydrolyses lactose and glucose + galactose is formed. Glucose is measured after a modified version of the common glucose oxidase / peroxidase assay (Werner, W. et al. (1970) Z. analyt. Chem. 252: 224.)

pH profile

Substrate:

[0127] 167 mM lactose, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and pH adjusted to pH 3, 4, 5, 6, 7, 8, 9 and 10 with NaOH.

Enzyme Sample:

[0128] Experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 was diluted appropriately in 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 0.01% Triton X100.

Procedure:

[0129]

- 10 µl enzyme sample diluted in enzyme dilution buffer was added to PCR tubes at room temp.
- 90 µl substrate was added at room temp. and quickly placed in a Peltier Thermal Cycler (PCT-200, MJ research) at 37°C and incubated for 30 min and then placed on ice.
- The reaction was stopped by adding 100 µl 0.25 M NaOH.
- 20 µl was transferred to a 96 well microtitre plate and 230 µl 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP, 0.65 g/l ABTS solution was added. After 30 minutes at room temperature, the absorbance was measured at 420 nm.

Table 3:

pH	<i>B. bifidum</i> lactase relative activity (% of activity at pH6)
3	0
4	4
5	75
6	100
7	85
8	39
9	10
10	4

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Temperature profile

Substrate:

- 5 **[0130]** 167 mM lactose, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and pH adjusted to pH 6.5 with NaOH.

Enzyme Sample:

- 10 **[0131]** Experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 was diluted appropriately in 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 0.01% Triton X100 and pH adjusted to pH 6.5.

Procedure:

- 15 **[0132]**
- 10 µl enzyme sample diluted in enzyme dilution buffer was added to PCR tubes at room temp.
 - 90 µl preheated (in a Peltier Thermal Cycler 30-70°C) substrate was added and incubation was performed with a temp. gradient from 30-70°C for 30 min. and then placed on ice.
 - The reaction was stopped by adding 100 µl 0.25 M NaOH.
 - 20 µl was transferred to a 96 well microtitre plate and 230 µl 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP, 0.65 g/l ABTS solution was added. After 30 minutes at room temperature, the absorbance was measured at 420 nm.

Table 4:

Temp. °C	<i>B. bifidum</i> lactase relative activity (% of activity at 38.1 °C)
20	54
21	63
22	64
24	68
26	73
29	81
31	88
34	94
36	96
38	100
43	96
48	91
52	83
57	76
62	58
66	32
69	20
70	17

Example 4*Determination of Km for lactase enzymes at 5°C*5 Principle:

[0133] Lactase hydrolyses lactose and glucose + galactose is formed. Glucose is measured after a modified version of the common glucose oxidase / peroxidase assay (Werner, W. et al. (1970) Z. analyt. Chem. 252: 224.)

10 Substrate:

[0134] Different lactose concentrations ranging from Km/5 to 10* Km, 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and pH adjusted to pH 6.5 with NaOH.

15 Enzyme Sample:

[0135] Experimental lactase from *Bifidobacterium bifidum* having the encoded sequence shown in SEQ ID NO: 2 was diluted appropriately in 50 mM succinate, 50 mM HEPES, 50 mM CHES, 150 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 0.01% Triton X100, pH adjusted to pH 6.5 with NaOH.

20 **[0136]** 12 g/l Lactozym (commercially available lactase from Novozymes A/S, Denmark) was diluted 6000 times in the same buffer.

Procedure:25 **[0137]**

- 10 µl enzyme sample (5°C) was added to a 96 well microtitre plate on ice.
- 90 µl substrate (5°C) was added and incubated for 2 hours at 5°C.
- The reaction was stopped by adding 100 µl 0.25 M NaOH.
- 30 - 20 µl was transferred to a 96 well microtitre plate and 230 µl 65 mM sodium phosphate, pH 7, 0.4 g/l Glucose oxidase, 0.013 g/l HRP, 0.65 g/l ABTS solution was added. After 30 minutes at room temperature, the absorbance was measured at 420 nm.

Km determination:

35

[0138] Computerized nonlinear least-squares fitting and the Michaelis-Menten equation:

$$v = (V_{max} * S) / (K_m + S)$$

40

was used. Km for the *Bifidobacterium* lactase and Lactozym were determined to be 8 mM and 30 mM, respectively.

[0139] In a similar test performed at 37°C, Km for the *Bifidobacterium* lactase and Lactozym were determined to be 13 mM and 30 mM, respectively.

45 **Example 5***Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - Different levels of sucrose and lactase*50 Experimental plan

[0140] For the selected combination of a low pH stable lactase and a lactose-deficient starter culture, two levels of added sucrose (0.5 % and 0.7 %), and two levels of added lactase (800 LAU/L and 1000 LAU/L), were tested. As reference fermentations with no added lactase and two levels of sucrose (1.0 % and 1.5 %) were carried out. The lactase was added at the start of the fermentation together with the starter culture.

55

Milk base**[0141]**

Table 5: Composition of milk base

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Skim milk	1000	3.5	4.8	0.1
1.5 % milk	2000	3.4	4.7	1.5
Protein powder "Promilk 802FB"	14	80.0		
Protein powder "Milex 240"	95	34.0		
Total milk base	3109	4.71	4.57	1.00

Starter culture

[0142] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0143] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Measurements

[0144] Fat, protein and lactose levels were determined using MilkoScan analysis. Post-acidification was measured over a period of 28 days storage at 5 °C.

[0145] The level of sucrose, glucose, galactose, fructose and lactose in the fermented milk at day 1 and day 14 after production was measured by HPLC.

Gel firmness

[0146] Gel firmness is measured by a back extrusion test with a texture analyzer (TA.XT Plus, Stable Micro System, Surrey, UK) supplied with a 35mm parallel plate. The travel distance is set to 15 mm, and the travel speed to 2 mm/s. The test is performed after 7 days from production. The fermented milk product was brought to 13°C and manually stirred gently, and measured in a 250 g plastic container. The maximal force (g) obtained by force versus distance curves is used as "gel firmness" parameter.

Shear stress

[0147] Seven days after incubation, the fermented milk product was brought to 13°C and manually stirred gently by means of a spoon (5 times) until homogeneity of the sample. The rheological properties of the sample were assessed on a rheometer (Anton Paar Physica Rheometer with ASC, Automatic Sample Changer, Anton Paar® GmbH, Austria) by using a bob-cup. The rheometer was set to a constant temperature of 13 °C during the time of measurement. Settings were as follows:

Holding time (to rebuild to somewhat original structure)

5 minutes without any physical stress (oscillation or rotation) applied to the sample. Oscillation step (to measure the elastic and viscous modulus, G' and G'', respectively, therefore calculating the complex modulus G*)

Constant strain = 0.3 %, frequency (f) = [0.5..8] Hz

6 measuring points over 60 s (one every 10 s)

Rotation step (to measure shear stress at 300 1/s)

Two steps were designed:

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1) Shear rate = [0.3-300] 1/s and 2) Shear rate = [275-0.3] 1/s.

Each step contained 21 measuring points over 210 s (on every 10 s).

The shear stress at 300 1/s was chosen for further analysis, as this correlates to mouth thickness when swallowing a fermented milk product.

Procedure

[0148] The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 5 °C. The milk base was then pasteurized at 90 °C for 20 minutes. The fermentation was carried out at 43 °C to an end pH of 4.55 to form yogurt. The yogurt was cooled in a PTU (Post Treatment Unit) at a cooling temperature of 25 °C at 2 bars. The cooled yogurt was stored at 6 °C.

Results

Post-acidification

[0149]

Table 6: Post-acidification

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%)	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Ferment. time (Hours: Min.)	7:25	7:05	7:15	7:15	7:00	7:00
End pH	4.58	4.55	4.55	4.55	4.55	4.55
pH Day 1	4.58	4.53	4.54	4.53	4.50	4.51
pH Day 7	4.54	4.49	4.50	4.50	4.47	4.49
pH Day 14	4.49	4.43	4.49	4.49	4.46	4.47
pH Day 21	4.35	4.34	4.45	4.45	4.42	4.43
pH Day 28	4.35	4.34	4.45	4.42	4.43	4.42
pH drop	0.23	0.19	0.09	0.11	0.07	0.09

[0150] For all six samples, i.e. 2 reference samples and 4 samples produced according to the invention (test samples), a pH of about 4.55 was reached in 7 hours.

[0151] As will appear from Table 6, the post-acidification over a period of 28 days was strongly reduced for the samples produced according to the invention as compared to the reference samples.

Carbohydrate analysis

[0152]

Table 7: Residual carbohydrate levels after 1 day from production

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%) start ferment.	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Fructose (mg/g)	1.62	1.91	0.80*	0.74*	1.27	1.18
Galactose (mg/g)	< 0.9	1.78*	29.01	29.87	28.78	29.29

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(continued)

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Glucose (mg/g)	0.77*	1.73	27.60	27.84	28.08	28.19
Lactose (mg/g)	54.16	53.37	1.18	0.40	1.20	0.40
Sucrose (mg/g)	< 0.3	4.6	< 0.3	< 0.3	1.3	0.7
*value is between Limit of Detection and Limit of Quantification.						

[0153] All levels of Table 7 are mean values of two samples.

[0154] For the 4 test samples, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference samples indicating high activity of the added lactase. For the test samples using a lactase level of 1000 LAU/L a residual lactose level of approx. 0.04 % was obtained, and for the test samples using a lactase level of 800 LAU/L a residual lactose level of approx. 0.1 % was obtained.

[0155] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of perceived sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness index than lactose.

Gel firmness

[0156]

Table 8: Gel firmness

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%) start ferment.	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Gel firmness (g)	45.76	47.42	48.19	54.57	50.19	46.60

[0157] All levels of Table 8 are mean values of two samples.

[0158] As will appear from Table 8, 3 out of the 4 test samples had significantly higher gel firmness than the 2 reference samples.

Shear stress

[0159]

Table 9: Shear stress measured at 300 s⁻¹

	Ref. 1	Ref. 2	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Sucrose (%) start ferment.	1	1.5	0.5	0.5	0.7	0.7
Lactase (LAU/ L)	0	0	800	1000	800	1000
Shear stress (Pa)	67.1	65.3	78.2	84.4	84.7	77.6

[0160] All levels of Table 9 are mean values of two samples.

[0161] As will appear from Table 9, the 4 test samples had significantly higher shear stress measured at 300 s⁻¹ than the 2 reference samples. In the group of 4 test samples, the combinations of 1) a level of added sucrose of 0.5 % and a lactase level of 1000 LAU/L, and 2) a level of added sucrose of 0.7 % and a lactase level of 800 LAU/L, had the highest shear stress.

Example 6

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of excess level of sucrose before fermentation I

Experimental plan

[0162] Fermentations were carried out with a reference sample containing no lactase and 9.5 % sucrose and 2 test samples containing 800 LAU/L of lactase and 6.5 % and 7.0 % sucrose. The lactase was added at the start of the fermentation together with the starter culture.

Milk base**[0163]**

Table 10: Composition of basic milk base 1

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Skim milk	984	3.5	4.8	0.1
1.5 % milk	1911	3.4	4.7	1.5
Protein powder "Promilk 802FB"	13.3	80.0		
Protein powder "Milex 240"	91.5	34.0	54.00	1.00
Total milk base	3000	4.71	4.57	1.02

Table 11: Composition of basic milk base 2

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Skim milk	984	3.5	4.8	0.1
1.5 % milk	1911	3.4	4.7	1.5
Protein powder "Promilk 802FB"	17.40	80.0		
Protein powder "Milex 240"	109.50	34.0	54.00	1.00
Sucrose	180.00		100.0	
Total milk base	3202	4.70	11.75	0.96

[0164] Basic milk base 2 was used to prepare the final milk bases for the reference sample and the two test samples by addition of additional sucrose.

Starter culture

[0165] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0166] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Measurements

[0167] All measurements were carried out using the same methods as in Example 5.

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Procedure

[0168] The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 5 °C. The milk base was then pasteurized at 95 °C for 5 minutes. The milk base was then homogenized at 65 °C at 200 bar. The fermentation was carried out at 43 °C to an end pH of 4.55 to form yogurt. The yogurt was cooled in a PTU (Post Treatment Unit) at a cooling temperature of 25 °C at 2 bars. The cooled yogurt was stored at 6 °C.

Results

Post-acidification

[0169]

Table 12: Post-acidification

Sample	1	2	3
Lactase (LAU/L)	0	800	800
Fermentation time	6 h 15 min	6 h 15 min	6 h 15 min
End pH	4.55	4.51	4.51
pH Day 4	4.54	4.44	4.44
pH Day 7	4.51	4.42	4.42
pH Day 14	4.40	4.38	4.37
pH Day 21	4.38	4.38	4.37
pH Day 28	4.36	4.37	4.36
pH drop	0.19	0.14	0.15

Carbohydrate analysis

[0170]

Table 13: Carbohydrate analysis

Sample	1		2		3	
Lactase (LAU/L)	0		800		800	
Sucrose (%) start ferment.	9.5		6.5		7.0	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Fructose (mg/g)	2.4	3.07	1.7	2.01	1.9	2.00
Galactose (mg/g)	< 2.0	8.99	26.4	29.03	26.4	28.72
Glucose (mg/g)	< 1.0	8.84	26.2	27.52	26.2	27.22
Lactose (mg/g)	51.4	38.04	2.8	< 2	2.7	< 2
Sucrose (mg/g)	64.3	64.23	41.7	42.38	46.0	46.26

[0171] All levels of Table 13 are mean values of two samples.

[0172] As will appear from Table 13, for the 2 test samples, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference sample indicating high activity of the added lactase.

[0173] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness than lactose.

*Gel firmness and shear stress***[0174]**Table 14: Gel firmness and shear stress at 300 s⁻¹

Sample	1		2		3	
Lactase (LAU/L)	0		800		800	
Sucrose (%) start ferment.	9.5		6.5		7.0	
Gel firmness (g)	46.36		46.76		45.75	
Shear stress at 1 s ⁻¹ (Pa)	9.4	9.2	9.3	8.8	10.3	10.2
Shear stress at 30.2 s ⁻¹ (Pa)	39.2	38.8	41.6	39.2	44.7	45.1
Shear stress at 135 s ⁻¹ (Pa)	58.3	58.0	67.2	64.5	72.3	72.2
Shear stress at 300 s ⁻¹ (Pa)	69.4	69.1	80.6	78.2	84.4	84.4

[0175] As will appear from Table 14, the shear stress at 300 s⁻¹ of the two test samples containing lactase was significantly increased as compared to the reference sample with no lactase.

Example 7

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of excess level of sucrose before fermentation II

Experimental plan

[0176] Fermentations were carried out with two reference samples containing no lactase and 1.5 % and 9.5 % sucrose and 3 test samples containing 1000 LAU/L of lactase and 0.5 %, 6.5 % and 7.0 % sucrose. The lactase was added at the start of the fermentation together with the starter culture.

Milk base

[0177] The two basic milk bases of Example 6 were used to prepare the final milk bases for the reference samples and the three test samples by addition of additional sucrose.

Starter culture

[0178] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0179] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Measurements

[0180] All measurements were carried out using the same methods as in Example 5.

Procedure

[0181] The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 5 °C. The milk base was then pasteurized at 90 °C for 20 minutes. The fermentation was carried out at 43 °C to an end pH of 4.55 to form yogurt. The yogurt was cooled in a PTU (Post Treatment Unit) at a cooling temperature of 25 °C at 2 bars. The cooled yogurt was stored at 6 °C.

Results*Post-acidification*

[0182]

Table 15: Post-acidification

Sam ple	1	2	3	4	5
Lactase (LAU/L)	0	1000	0	1000	1000
Fermentation time	6 h 50 min	6 h 55 min	6 h 35 min	6 h 10 min	5 h 40 min
End pH	4.55*	4.55*	4.55*	4.55*	4.55*
pH Day 1	4.54	4.51	4.52	4.48	4.47
pH Day 7	4.45	4.45	4.40	4.34	4.35
pH Day 14	4.37	4.44	4.37	4.32	4.34
pH Day 21	4.35	4.39	4.35	4.29	4.29
pH Day 28	4.34	4.37	4.33	4.27	4.26
pH drop	0.21	0.18	0.22	0.28	0.29
*The fermentation is stopped by cooling at pH = 4.55.					

[0183] As will appear from Table 15, the post-acidification was at the same level for the reference samples containing no lactase and the test samples containing lactase.

Carbohydrate analysis

[0184]

Table 16: Carbohydrate analysis Day 1

Sam ple	1	2	3	4	5
Lactase (LAU/ L)	0	1000	0	1000	1000
Sucrose (%) start ferment.	1.5	0.5	9.5	6.5	7.0
Fructose (mg/g)	1.96	< 1	2.70	1.74	1.88
Galactose (mg/g)	< 2	29.19	< 2	27.83	27.89
Glucose (mg/g)	< 1	27.37	< 1	27.29	27.40
Lactose (mg/g)	56.11	< 2	54.56	< 2	< 2
Sucrose (mg/g)	5.45	< 4	63.44	39.79	45.44

Table 17: Carbohydrate analysis Day 28

Sam ple	1	2	3	4	5
Lactase (LAU/ L)	0	1000	0	1000	1000
Sucrose (%) start ferment.	1.5	0.5	9.5	6.5	7.0
Fructose (mg/g)	1.88	< 1	4.72	2.95	3.39
Galactose (mg/g)	3.90	28.83	< 2	28.79	29.15
Glucose (mg/g)	2.69	26.78	1.66	26.83	27.27
Lactose (mg/g)	50.71	< 2	52.42	< 2	< 2

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(continued)

Sam ple	1	2	3	4	5
Sucrose (mg/g	4.18	< 4	62.07	39.91	46.17

[0185] As will appear from Tables 16 and 17, for the 3 test samples, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference sample indicating high activity of the added lactase.

[0186] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness than lactose.

Gel firmness and shear stress

[0187]

Table 18: Gel firmness and shear stress

Sam ple	1		2		3		4		5	
Lactase (LAU/L)	0		1000		0		1000		1000	
Sucrose (%) start ferment.	1.5		0.5		9.5		6.5		7.0	
Gel firmness (g)	51.13		49.41		45.04		45.94		47.89	
Shear stress at 1 s-1 (Pa)	9.4	9.3	8.4	8.5	8.1	8.5	7.7	7.8	8.3	8.5
Shear stress at 30.2 s-1 (Pa)	39.4	38.5	38.2	37.6	34.3	34.7	37.3	37.1	38.8	38.9
Shear stress at 135 s-1 (Pa)	61.0	59.5	65.8	65.1	56.8	56.8	72.7	72.3	74.8	74.9
Shear stress at 300 s-1 (Pa)	72.1	70.5	78.8	77.6	69.1	69.1	86.7	86.6	89.4	89.4

[0188] As will appear from Table 18, the shear stress of the three test samples containing lactase was significantly increased as compared to the reference sample with no lactase.

Example 8

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of lactase before and after fermentation

Experimental plan

[0189]

Table 19: Experimental plan

Sample	Timing of lactase addition	Milk Base	Sucrose (%)	Lactase (LAU/L)
1	None	1	0.70	0
2	Together with culture	1	0.70	600
3	Together with culture	1	0.70	800
4	Together with culture	1	0.70	1000
5	Together with culture	1	0.70	1400
6	None	2	0.97	0
7	After fermentation	2	0.97	800
8	After fermentation	2	0.97	1600

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(continued)

Sample	Timing of lactase addition	Milk Base	Sucrose (%)	Lactase (LAU/L)
9	After fermentation	2	0.97	2400
10	After fermentation	2	0.97	3200

[0190] The fermentation temperature was 43 °C. The end pH was 4.50. The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 6 °C. The milk base was then pasteurized at 95 °C for 5 minutes and homogenized at 200/50 bar at 65 °C. The fermentation was carried out at 43 °C to an end pH of 4.50 to form yogurt. The yogurt was cooled to 6 °C. The cooled yogurt was stored at 6 °C.

Starter culture

[0191] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0192] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Milk base

[0193]

Table 20: Composition of milk base 1

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Sucrose	22	0.0	100.0	0.0
0.5 % milk	1500	3.8	4.8	0.5
1.5 % milk	1500	3.6	4.7	1.5
Arla Skimmed Milk Powder	103	34.0	54.0	1.0
Total milk base	3125	4.67	7.05	0.98

Table 21: Composition of milk base 2

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
Sucrose	30	0.0	100.0	0.0
0.5 % milk	1500	3.8	4.8	0.5
1.5 % milk	1500	3.6	4.7	1.5
Arla Skimmed Milk Powder	103	34.0	54.0	1.0
Total milk base	3133	4.66	7.30	1.00

Measurements

[0194] All measurements were carried out using the same methods as in Example 5.

Results

Post-acidification

[0195]

Table 22: Post-acidification

Sample	Timing of lactase addition	Milk Base	Lactase (LAU/L)	pH Day 0	pH Day 7	pH Day 28	pH Day 42	pH drop
1	None	1	0	4.89*	4.89*	4.87	4.84	0.05
2	Together with culture	1	600	4.26	4.23	4.23	4.22	0.04
3	Together with culture	1	800	4.22	4.26	4.24	4.24	-0.02
4	Together with culture	1	1000	4.32	4.26	4.26	4.26	0.06
5	Together with culture	1	1400	4.36	4.30	4.28	4.27	0.09
6	None	2	0	4.47	4.47	4.47	4.44	0.03
7	After fermentation	2	800	4.43	4.40	4.42	4.38	0.05
8	After fermentation	2	1600	4.44	4.40	4.42	4.39	0.05
9	After fermentation	2	2400	4.41	4.43	4.40	4.40	0.01
10	After fermentation	2	3200	NA	4.43	4.41	4.40	NA
* Insufficient sucrose to obtain fermentation to target pH. Therefore, this sample cannot be used as reference.								

[0196] As will appear from Table 22, the post-acidification over a period of 42 days was at a low level of below 0.09 for all lactase levels tested with lactase addition at the start of fermentation and at an even lower level of below 0.05 for all lactase levels tested with lactase addition at the end of fermentation. The addition of lactase at the end of the fermentation did not result in a statistically different post-acidification as compared to the reference sample with no added lactase. Thus, the present experiment shows that the use of a low pH stable lactase in combination with a lactose-deficient starter culture does not result in an unacceptable increase of the low post-acidification, which may be obtained with such a starter culture. This is true both when the lactase is added at the start and at the end of fermentation.

Gel firmness and shear stress

[0197]

Table 23: Gel firmness and Shear Stress

Sample	Timing of lactase addition	Milk Base	Sucrose (%)	Lactase (LAU/L)	Gel firmness (g)	Shear Stress (Pa)
1	None	1	0.70	0	0.187*	46.4*
2	Together with culture	1	0.70	600	0.281	63.9
3	Together with culture	1	0.70	800	0.302	65.3
4	Together with culture	1	0.70	1000	0.318	64.5
5	Together with culture	1	0.70	1400	0.307	63.7
6	None	2	0.97	0	0.304	53.1
7	After fermentation	2	0.97	800	0.312	51.4
8	After fermentation	2	0.97	1600	0.322	52.8
9	After fermentation	2	0.97	2400	0.323	52.9

(continued)

Sample	Timing of lactase addition	Milk Base	Sucrose (%)	Lactase (LAU/L)	Gel firmness (g)	Shear Stress (Pa)
10	After fermentation	2	0.97	3200	0.320	51.7
* Insufficient sucrose to obtain fermentation to target pH. Therefore, this sample cannot be used as reference.						

[0198] As will appear from Table 23, high levels of both gel firmness and shear stress was obtained for all lactase levels tested with lactase addition at the start of fermentation. For lactase levels tested with lactase addition at the end of fermentation, the levels of gel firmness and shear stress are somewhat lower, which is due to the mixing of the lactase into the yogurt, which partly disrupts the texture of the yogurt. For all lactase levels tested with lactase addition at the end of fermentation, the gel firmness is slightly higher than the reference sample with no added lactase. For all lactase levels tested with lactase addition at the end of fermentation, the shear stress is maintained at the same level or slightly lower.

Carbohydrate analysis

[0199]

Table 24: Carbohydrate analysis

Sam-ple	Lactase (LAU/L)	Galactose Day 20 (mg/g)	Glucose Day 20 (mg/g)	Sucrose Day 20 (mg/g)	Lactose Day 20 (mg/g)	Lactose 24 hours (%)	Lactose Day 20 (%)
1	0	2.4	2.3	< 1.0	50.7	ND	ND
2	600	29.5	27.7	< 1.0	< 0.5	> 0.1	0.023
3	800	30.2	28.1	< 1.0	< 0.5	0.028	0.016
4	1000	30.0	27.8	< 1.0	< 0.5	0.014	0.014
5	1400	29.5	27.4	< 1.0	< 0.5	0.006	0.013
6	0	4.2	4.2	< 1.0	49.2	ND	ND
7	800	31.2	30.7	< 1.0	< 0.5	> 0.2	0.014
8	1600	30.7	30.2	< 1.0	< 0.5	> 0.1	0.012
9	2400	31.2	30.6	< 1.0	< 0.5	0.029	0.011
10	3200	31.8	31.2	< 1.0	< 0.5	0.015	0.011

[0200] As will appear from Table 24, at Day 20 high concentrations of galactose and glucose are formed for all lactase levels tested both with lactase addition at the start and at the end of fermentation. Also, at Day 20 the level of glucose was below the detection limit of 0.5 mg/g, which qualifies as lactose free in some countries. For the higher levels of lactase, most of the lactose removal has been obtained 24 hours after the end of fermentation.

Example 9

Production of yogurt using lactase from Bifidobacterium bifidum and a lactose-deficient starter culture - addition of lactase before fermentation II

Experimental plan

[0201]

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Table 25: Experimental plan

Sample	Sucrose (%)	Lactase (LAU/L)
1	0	0
2	0	1000
3	0	1200
4	0	1600
5	0.2	0
6	0.2	1000
7	0.2	1200
8	0.2	1600
9	0.7	0
10	0.7	1000
11	0.7	1200
12	0.7	1600

[0202] The fermentation temperature was 43 °C. The end pH was 4.45. The ingredients of the milk base were mixed and allowed to re-hydrate for 2 hours at 6 °C. The milk base was then pasteurized at 95 °C for 5 minutes and homogenized at 200/50 bar at 65 °C. The fermentation was carried out at 43 °C to an end pH of 4.45 to form yogurt. The yogurt was cooled to 5 °C. The cooled yogurt was stored at 6 °C.

Starter culture

[0203] The starter culture is composed of *Streptococcus thermophilus* strain deposited under the accession no. DSM 28952, *Streptococcus thermophilus* strain deposited under the accession no. DSM 28953, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strain deposited under accession no. DSM 28910.

Lactase

[0204] Lactase from *Bifidobacterium bifidum* having the encoded sequence of SEQ ID NO. 2.

Milk base

[0205]

Table 26: Composition of milk base

	Amount (g)	Protein content (%)	Carbohydrate (%)	Fat (%)
0.5 % milk	1500	3.8	4.8	0.5
1.5 % milk	1500	3.6	4.7	1.5
Arla Skimmed Milk Powder	103	34.0	54.0	1.0
Total milk base	3103	4.70	6.39	0.99

Measurements

[0206] All measurements were carried out using the same methods as in Example 5.

Results*Post-acidification*

[0207]

Table 27: Post-acidification

Sample	Sucrose (%)	Lactase (LAU/L)	pH Day 0	pH Day 1	pH Day 14	pH Day 28	pH Day 42	pH drop
1	0	0	ND	ND	ND	ND	ND	ND
2	0	1000	4.45	4.39	4.36	4.33	4.39	0.06
3	0	1200	4.45	4.39	4.38	4.34	4.39	0.06
4	0	1600	4.46	4.39	4.38	4.34	4.39	0.07
5	0.2	0	ND	ND	ND	ND	ND	ND
6	0.2	1000	4.50	4.44	4.40	4.40	4.43	0.07
7	0.2	1200	4.53	4.47	4.46	4.42	4.46	0.07
8	0.2	1600	4.53	4.49	4.46	4.44	4.47	0.06
9	0.7	0	4.76	4.73	4.69	4.68	4.70	0.06
10	0.7	1000	4.44	4.40	4.39	4.33	4.38	0.06
11	0.7	1200	4.46	4.40	4.39	4.33	4.39	0.07
12	0.7	1600	4.47	4.41	4.40	4.34	4.41	0.06

[0208] As will appear from Table 27, the post-acidification over a period of 42 days was at a low very level of approx. 0.06 for all lactase and sucrose levels tested. Thus, the present experiment shows that the use of a low pH stable lactase in combination with a lactose-deficient starter culture does not result in an unacceptable increase of the low post-acidification, which may be obtained with such a starter culture.

Gel firmness and shear stress

[0209]

Table 28: Gel firmness and Shear Stress

Sample	Sucrose (%)	Lactase (LAU/L)	Gel firmness (g)	Shear Stress (Pa)
1	0	0	ND	ND
2	0	1000	0.386	70.3
3	0	1200	0.377	69.8
4	0	1600	0.388	69.5
5	0.20	0	ND	ND
6	0.20	1000	0.380	70.7
7	0.20	1200	0.378	69.5
8	0.20	1600	0.397	70.2
9	0.70	0	ND	ND
10	0.70	1000	0.362	71.2
11	0.70	1200	0.375	72.2
12	0.70	1600	0.362	71.9

[0210] As will appear from Table 28, high levels of both gel firmness and shear stress was obtained for all lactase levels tested. The gel firmness and shear strength are at the same order of magnitude for all three levels of lactase tested.

Carbohydrate analysis

[0211]

Table 29: Carbohydrate analysis

Sam-ple	Sucrose (%)	Lactase (LAU/L)	Fructose (mg/g)	Galactose (mg/g)	Glucose (mg/g)	Lactose (mg/g)	Sucrose (mg/g)
1	0	0	< 0.5	6.3	< 0.5	45.5	<2
2	0	1000	< 0.5	30.7	26.0	< 0.9	<2
3	0	1200	< 0.5	31.5	26.6	< 0.9	<2
4	0	1600	< 0.5	30.9	25.8	< 0.9	<2
5	0.20	0	< 0.5	11.7	6.7	36.1	<2
6	0.20	1000	< 0.5	30.8	27.8	< 0.9	<2
7	0.20	1200	< 0.5	31.6	28.3	< 0.9	<2
8	0.20	1600	< 0.5	31.5	28.0	< 0.9	<2
9	0.70	0	1.1	9.1	9.1	43.4	<2
10	0.70	1000	1.1	31.1	29.5	< 0.9	<2
11	0.70	1200	1.0	32.1	30.3	< 0.9	1.8
12	0.70	1600	0.9	31.8	29.9	< 0.9	1.9

[0212] As will appear from Table 29, for the test samples containing lactase, at the end of fermentation the level of lactose was very low and the level of glucose and galactose was very high as compared to the reference sample indicating high activity of the added lactase.

[0213] Fermented milk having a low lactose level and a high level of glucose and galactose has a much higher level of sweetness than fermented milk with a high lactose level and a low level of glucose and galactose like the reference samples. The reason for this is that glucose and galactose has a much higher sweetness than lactose.

Sequence listing

[0214]

SEQ ID NO.: 1 shows the sequence of a mutant of SEQ ID NO. 4.

SEQ ID NO.: 2 shows the sequence of a mutant of SEQ ID NO. 4.

SEQ ID NO.: 3 shows the sequence of a lactase from *Bifidobacterium bifidum* DSM20215.

SEQ ID NO.: 4 shows the sequence of a lactase from *Bifidobacterium bifidum* NCIMB41171, the nucleotide sequence of which is listed in NCBI with the accession number DQ448279.

[0215] SEQ ID NO: 4 is discussed in the following references, wherein it is referred to as bbgIII:

Appl Microbiol Biotechnol (2007) 76:1365-1372, T K Goulas et al.

Appl Microbiol Biotechnol (2009) 82: 1079-1088, T Goulas et al.

Appl Microbiol Biotechnol (2009) 84:899-907, T Goulas et al.

SEQUENCE LISTING

[0216]

<110> Chr. Hansen A/S

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<120> Process for producing a fermented milk product

<130> P6170PC00

5 <160> 4

<170> PatentIn version 3.5

<210> 1

10 <211> 1931

<212> PRT

<213> Bifidobacterium bifidum

<400> 1

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20 25 30

25

Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr Pro Glu Val Ala
35 40 45

30

Tyr Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr Ser Asp Phe Asp
50 55 60

Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln Ala Gln Asp Pro
65 70 75 80

35

Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu Pro His Asp Tyr
85 90 95

40

Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala Glu Ser Ala Tyr
100 105 110

45

Leu Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe Thr Ile Asp Arg
115 120 125

Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp Gly Val Tyr Met
130 135 140

50

Asn Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly Thr His Pro Tyr
145 150 155 160

55

Gly Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn Ala Lys Phe Gly
165 170 175

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	Gly	Glu	Asn	Thr	Ile	Val	Val	Lys	Val	Glu	Asn	Arg	Leu	Pro	Ser	Ser	
				180					185					190			
5	Arg	Trp	Tyr	Ser	Gly	Ser	Gly	Ile	Tyr	Arg	Asp	Val	Thr	Leu	Thr	Val	
			195					200					205				
10	Thr	Asp	Gly	Val	His	Val	Gly	Asn	Asn	Gly	Val	Ala	Ile	Lys	Thr	Pro	
		210					215					220					
15	Ser	Leu	Ala	Thr	Gln	Asn	Gly	Gly	Asp	Val	Thr	Met	Asn	Leu	Thr	Thr	
	225					230					235					240	
20	Lys	Val	Ala	Asn	Asp	Thr	Glu	Ala	Ala	Ala	Asn	Ile	Thr	Leu	Lys	Gln	
					245					250					255		
25	Thr	Val	Phe	Pro	Lys	Gly	Gly	Lys	Thr	Asp	Ala	Ala	Ile	Gly	Thr	Val	
				260					265					270			
30	Thr	Thr	Ala	Ser	Lys	Ser	Ile	Ala	Ala	Gly	Ala	Ser	Ala	Asp	Val	Thr	
			275					280					285				
35	Ser	Thr	Ile	Thr	Ala	Ala	Ser	Pro	Lys	Leu	Trp	Ser	Ile	Lys	Asn	Pro	
	290						295					300					
40	Asn	Leu	Tyr	Thr	Val	Arg	Thr	Glu	Val	Leu	Asn	Gly	Gly	Lys	Val	Leu	
	305					310					315					320	
45	Asp	Thr	Tyr	Asp	Thr	Glu	Tyr	Gly	Phe	Arg	Trp	Thr	Gly	Phe	Asp	Ala	
					325					330					335		
50	Thr	Ser	Gly	Phe	Ser	Leu	Asn	Gly	Glu	Lys	Val	Lys	Leu	Lys	Gly	Val	
				340					345					350			
55	Ser	Met	His	His	Asp	Gln	Gly	Ser	Leu	Gly	Ala	Val	Ala	Asn	Arg	Arg	
			355					360					365				
60	Ala	Ile	Glu	Arg	Gln	Val	Glu	Ile	Leu	Gln	Lys	Met	Gly	Val	Asn	Ser	
		370					375					380					
65	Ile	Arg	Thr	Thr	His	Asn	Pro	Ala	Ala	Lys	Ala	Leu	Ile	Asp	Val	Cys	
	385					390					395					400	
70	Asn	Glu	Lys	Gly	Val	Leu	Val	Val	Glu	Glu	Val	Phe	Asp	Met	Trp	Asn	
					405					410					415		
75	Arg	Ser	Lys	Asn	Gly	Asn	Thr	Glu	Asp	Tyr	Gly	Lys	Trp	Phe	Gly	Gln	
				420					425					430			

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	Ala	Ile	Ala	Gly	Asp	Asn	Ala	Val	Leu	Gly	Gly	Asp	Lys	Asp	Glu	Thr	
			435					440					445				
5	Trp	Ala	Lys	Phe	Asp	Leu	Thr	Ser	Thr	Ile	Asn	Arg	Asp	Arg	Asn	Ala	
		450					455					460					
	Pro	Ser	Val	Ile	Met	Trp	Ser	Leu	Gly	Asn	Glu	Met	Met	Glu	Gly	Ile	
10		465				470					475					480	
	Ser	Gly	Ser	Val	Ser	Gly	Phe	Pro	Ala	Thr	Ser	Ala	Lys	Leu	Val	Ala	
					485					490					495		
15	Trp	Thr	Lys	Ala	Ala	Asp	Ser	Thr	Arg	Pro	Met	Thr	Tyr	Gly	Asp	Asn	
				500					505					510			
	Lys	Ile	Lys	Ala	Asn	Trp	Asn	Glu	Ser	Asn	Thr	Met	Gly	Asp	Asn	Leu	
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	Thr	Ala	Asn	Gly	Gly	Val	Val	Gly	Thr	Asn	Tyr	Ser	Asp	Gly	Ala	Asn	
		530					535						540				
25	Tyr	Asp	Lys	Ile	Arg	Thr	Thr	His	Pro	Ser	Trp	Ala	Ile	Tyr	Gly	Ser	
	545					550					555					560	
	Glu	Thr	Ala	Ser	Ala	Ile	Asn	Ser	Arg	Gly	Ile	Tyr	Asn	Arg	Thr	Thr	
30					565					570					575		
	Gly	Gly	Ala	Gln	Ser	Ser	Asp	Lys	Gln	Leu	Thr	Ser	Tyr	Asp	Asn	Ser	
35				580					585					590			
	Ala	Val	Gly	Trp	Gly	Ala	Val	Ala	Ser	Ser	Ala	Trp	Tyr	Asp	Val	Val	
			595					600					605				
40	Gln	Arg	Asp	Phe	Val	Ala	Gly	Thr	Tyr	Val	Trp	Thr	Gly	Phe	Asp	Tyr	
		610					615					620					
	Leu	Gly	Glu	Pro	Thr	Pro	Trp	Asn	Gly	Thr	Gly	Ser	Gly	Ala	Val	Gly	
45		625				630					635					640	
	Ser	Trp	Pro	Ser	Pro	Lys	Asn	Ser	Tyr	Phe	Gly	Ile	Val	Asp	Thr	Ala	
					645					650					655		
50	Gly	Phe	Pro	Lys	Asp	Thr	Tyr	Tyr	Phe	Tyr	Gln	Ser	Gln	Trp	Asn	Asp	
				660					665					670			
55	Asp	Val	His	Thr	Leu	His	Ile	Leu	Pro	Ala	Trp	Asn	Glu	Asn	Val	Val	
			675					680					685				

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	Ala	Lys	Gly	Ser	Gly	Asn	Asn	Val	Pro	Val	Val	Val	Tyr	Thr	Asp	Ala	
	690						695						700				
5	Ala	Lys	Val	Lys	Leu	Tyr	Phe	Thr	Pro	Lys	Gly	Ser	Thr	Glu	Lys	Arg	
	705					710					715					720	
10	Leu	Ile	Gly	Glu	Lys	Ser	Phe	Thr	Lys	Lys	Thr	Thr	Ala	Ala	Gly	Tyr	
					725					730					735		
15	Thr	Tyr	Gln	Val	Tyr	Glu	Gly	Ser	Asp	Lys	Asp	Ser	Thr	Ala	His	Lys	
				740					745					750			
20	Asn	Met	Tyr	Leu	Thr	Trp	Asn	Val	Pro	Trp	Ala	Glu	Gly	Thr	Ile	Ser	
			755					760					765				
25	Ala	Glu	Ala	Tyr	Asp	Glu	Asn	Asn	Arg	Leu	Ile	Pro	Glu	Gly	Ser	Thr	
		770					775					780					
30	Glu	Gly	Asn	Ala	Ser	Val	Thr	Thr	Thr	Gly	Lys	Ala	Ala	Lys	Leu	Lys	
	785					790					795					800	
35	Ala	Asp	Ala	Asp	Arg	Lys	Thr	Ile	Thr	Ala	Asp	Gly	Lys	Asp	Leu	Ser	
					805					810					815		
40	Tyr	Ile	Glu	Val	Asp	Val	Thr	Asp	Ala	Asn	Gly	His	Ile	Val	Pro	Asp	
				820					825					830			
45	Ala	Ala	Asn	Arg	Val	Thr	Phe	Asp	Val	Lys	Gly	Ala	Gly	Lys	Leu	Val	
			835					840					845				
50	Gly	Val	Asp	Asn	Gly	Ser	Ser	Pro	Asp	His	Asp	Ser	Tyr	Gln	Ala	Asp	
	850						855					860					
55	Asn	Arg	Lys	Ala	Phe	Ser	Gly	Lys	Val	Leu	Ala	Ile	Val	Gln	Ser	Thr	
	865					870					875					880	
60	Lys	Glu	Ala	Gly	Glu	Ile	Thr	Val	Thr	Ala	Lys	Ala	Asp	Gly	Leu	Gln	
					885					890					895		
65	Ser	Ser	Thr	Val	Lys	Ile	Ala	Thr	Thr	Ala	Val	Pro	Gly	Thr	Ser	Thr	
				900					905					910			
70	Glu	Lys	Thr	Val	Arg	Ser	Phe	Tyr	Tyr	Ser	Arg	Asn	Tyr	Tyr	Val	Lys	
			915					920					925				
75	Thr	Gly	Asn	Lys	Pro	Ile	Leu	Pro	Ser	Asp	Val	Glu	Val	Arg	Tyr	Ser	

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	Gly	Lys	Asn	Trp	Thr	Asp	Leu	Ala	Ala	Thr	Glu	Thr	Ile	Ala	Ala
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	1190						1195					1200			
10	Pro	Val	Gly	Ala	Thr	Phe	Val	Lys	Val	Thr	Val	Thr	Asn	Ala	Asp
	1205						1210					1215			
15	Thr	Thr	Thr	Pro	Ser	Gly	Val	Val	Cys	Ala	Gly	Leu	Thr	Glu	Ile
	1220						1225					1230			
20	Glu	Leu	Lys	Thr	Ala	Thr	Ser	Lys	Phe	Val	Thr	Asn	Thr	Ser	Ala
	1235						1240					1245			
25	Ala	Leu	Ser	Ser	Leu	Thr	Val	Asn	Gly	Thr	Lys	Val	Ser	Asp	Ser
	1250						1255					1260			
30	Val	Leu	Ala	Ala	Gly	Ser	Tyr	Asn	Thr	Pro	Ala	Ile	Ile	Ala	Asp
	1265						1270					1275			
35	Val	Lys	Ala	Glu	Gly	Glu	Gly	Asn	Ala	Ser	Val	Thr	Val	Leu	Pro
	1280						1285					1290			
40	Ala	His	Asp	Asn	Val	Ile	Arg	Val	Ile	Thr	Glu	Ser	Glu	Asp	His
	1295						1300					1305			
45	Val	Thr	Arg	Lys	Thr	Phe	Thr	Ile	Asn	Leu	Gly	Thr	Glu	Gln	Glu
	1310						1315					1320			
50	Phe	Pro	Ala	Asp	Ser	Asp	Glu	Arg	Asp	Tyr	Pro	Ala	Ala	Asp	Met
	1325						1330					1335			
55	Thr	Val	Thr	Val	Gly	Ser	Glu	Gln	Thr	Ser	Gly	Thr	Ala	Thr	Glu
	1340						1345					1350			
60	Gly	Pro	Lys	Lys	Phe	Ala	Val	Asp	Gly	Asn	Thr	Ser	Thr	Tyr	Trp
	1355						1360					1365			
65	His	Ser	Asn	Trp	Thr	Pro	Thr	Thr	Val	Asn	Asp	Leu	Trp	Ile	Ala
	1370						1375					1380			
70	Phe	Glu	Leu	Gln	Lys	Pro	Thr	Lys	Leu	Asp	Ala	Leu	Arg	Tyr	Leu
	1385						1390					1395			
75	Pro	Arg	Pro	Ala	Gly	Ser	Lys	Asn	Gly	Ser	Val	Thr	Glu	Tyr	Lys
	1400						1405					1410			

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5	Gly	Thr	Trp	Thr	Thr	Asp	Tyr	Gly	Trp	Lys	Leu	Ala	Glu	Phe	Asn
	1430						1435					1440			
10	Gln	Pro	Val	Thr	Thr	Lys	His	Val	Arg	Leu	Lys	Ala	Val	His	Thr
	1445						1450					1455			
15	Tyr	Ala	Asp	Ser	Gly	Asn	Asp	Lys	Phe	Met	Ser	Ala	Ser	Glu	Ile
	1460						1465					1470			
20	Arg	Leu	Arg	Lys	Ala	Val	Asp	Thr	Thr	Asp	Ile	Ser	Gly	Ala	Thr
	1475						1480					1485			
25	Val	Thr	Val	Pro	Ala	Lys	Leu	Thr	Val	Asp	Arg	Val	Asp	Ala	Asp
	1490						1495					1500			
30	His	Pro	Ala	Thr	Phe	Ala	Thr	Lys	Asp	Val	Thr	Val	Thr	Leu	Gly
	1505						1510					1515			
35	Asp	Ala	Thr	Leu	Arg	Tyr	Gly	Val	Asp	Tyr	Leu	Leu	Asp	Tyr	Ala
	1520						1525					1530			
40	Gly	Asn	Thr	Ala	Val	Gly	Lys	Ala	Thr	Val	Thr	Val	Arg	Gly	Ile
	1535						1540					1545			
45	Asp	Lys	Tyr	Ser	Gly	Thr	Val	Ala	Lys	Thr	Phe	Thr	Ile	Glu	Leu
	1550						1555					1560			
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	1565						1570					1575			
55	Lys	Thr	Lys	Pro	Ser	Lys	Leu	Thr	Tyr	Val	Val	Gly	Asp	Ala	Phe
	1580						1585					1590			
60	Asp	Pro	Ala	Gly	Leu	Val	Leu	Gln	Leu	Asn	Tyr	Asp	Asp	Asp	Ser
	1595						1600					1605			
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	1610						1615					1620			
70	Phe	Lys	Pro	Ala	Leu	Asp	Ala	Lys	Leu	Lys	Val	Thr	Asp	Lys	Thr
	1625						1630					1635			
75	Val	Thr	Val	Thr	Tyr	Gln	Gly	Lys	Ser	Ala	Val	Ile	Asp	Ile	Thr
	1640						1645					1650			

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	Val	Ser	Gln	Pro	Ala	Pro	Thr	Val	Ser	Lys	Thr	Asp	Leu	Asp	Lys
	1655						1660					1665			
5	Ala	Ile	Lys	Ala	Ile	Glu	Ala	Lys	Asn	Pro	Asp	Ser	Ser	Lys	Tyr
	1670						1675					1680			
10	Thr	Ala	Asp	Ser	Trp	Lys	Thr	Phe	Ala	Asp	Ala	Met	Ala	His	Ala
	1685						1690					1695			
15	Lys	Ala	Val	Ile	Ala	Asp	Asp	Ser	Ala	Thr	Gln	Gln	Asp	Val	Asp
	1700						1705					1710			
20	Asn	Ala	Leu	Lys	Ala	Leu	Thr	Asp	Ala	Tyr	Ala	Gly	Leu	Thr	Glu
	1715						1720					1725			
25	Lys	Thr	Pro	Glu	Pro	Ala	Pro	Val	Ser	Lys	Ser	Glu	Leu	Asp	Lys
	1730						1735					1740			
30	Lys	Ile	Lys	Ala	Ile	Glu	Ala	Glu	Lys	Leu	Asp	Gly	Ser	Lys	Tyr
	1745						1750					1755			
35	Thr	Ala	Glu	Ser	Trp	Lys	Ala	Phe	Glu	Thr	Ala	Leu	Ala	His	Ala
	1760						1765					1770			
40	Lys	Ala	Val	Ile	Ala	Ser	Asp	Ser	Ala	Thr	Gln	Gln	Asn	Val	Asp
	1775						1780					1785			
45	Ala	Ala	Leu	Gly	Ala	Leu	Thr	Ser	Ala	Arg	Asp	Gly	Leu	Thr	Glu
	1790						1795					1800			
50	Lys	Gly	Glu	Val	Lys	Pro	Asp	Pro	Lys	Pro	Glu	Pro	Gly	Thr	Val
	1805						1810					1815			
55	Asp	Lys	Ala	Ala	Leu	Asp	Lys	Ala	Val	Lys	Lys	Val	Glu	Ala	Glu
	1820						1825					1830			
60	Lys	Leu	Asp	Gly	Ser	Lys	Tyr	Thr	Ala	Asp	Ser	Trp	Lys	Ala	Phe
	1835						1840					1845			
65	Glu	Thr	Ala	Leu	Ala	His	Ala	Lys	Ala	Val	Ile	Gly	Asn	Ala	Asn
	1850						1855					1860			
70	Ser	Thr	Gln	Phe	Asp	Ile	Asp	Asn	Ala	Leu	Ser	Met	Leu	Asn	Asp
	1865						1870					1875			
75	Ala	Arg	Ala	Ala	Leu	Lys	Glu	Lys	Pro	Gly	Arg	Ile	Ile	Ala	Ile

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	1880					1885						1890			
5	Ile	Asp	Gly	Ser	Ala	Leu	Ser	Lys	Thr	Gly	Ala	Ser	Val	Ala	Ile
	1895						1900					1905			
10	Ile	Ala	Ser	Val	Ala	Ala	Ala	Met	Leu	Ala	Val	Gly	Ala	Gly	Val
	1910						1915					1920			
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	<211> 1341														
	<212> PRT														
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25															
30															
35															
40															
45															
50															
55															

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	Met	Lys	Lys	Pro	Leu	Gly	Lys	Ile	Val	Ala	Ser	Thr	Ala	Leu	Leu	Ile
	1				5					10					15	
5	Ser	Val	Ala	Phe	Ser	Ser	Ser	Ile	Ala	Ser	Ala	Ile	Glu	Asp	Ala	Thr
				20					25					30		
10	Arg	Ser	Asp	Ser	Thr	Thr	Gln	Met	Ser	Ser	Thr	Pro	Glu	Val	Ala	Tyr
			35					40					45			
15	Ser	Ser	Ala	Val	Asp	Ser	Lys	Gln	Asn	Arg	Thr	Ser	Asp	Phe	Asp	Ala
		50					55					60				
20	Asn	Trp	Lys	Phe	Met	Leu	Ser	Asp	Ser	Val	Gln	Ala	Gln	Asp	Pro	Ala
	65					70					75					80
25	Phe	Asp	Asp	Ser	Ala	Trp	Gln	Gln	Val	Asp	Leu	Pro	His	Asp	Tyr	Ser
					85					90					95	
30	Ile	Thr	Gln	Lys	Tyr	Ser	Gln	Ser	Asn	Glu	Ala	Glu	Ser	Ala	Tyr	Leu
				100					105					110		
35	Pro	Gly	Gly	Thr	Gly	Trp	Tyr	Arg	Lys	Ser	Phe	Thr	Ile	Asp	Arg	Asp
			115					120					125			
40	Leu	Ala	Gly	Lys	Arg	Ile	Ala	Ile	Asn	Phe	Asp	Gly	Val	Tyr	Met	Asn
		130					135					140				
45	Ala	Thr	Val	Trp	Phe	Asn	Gly	Val	Lys	Leu	Gly	Thr	His	Pro	Tyr	Gly
	145					150					155					160
50	Tyr	Ser	Pro	Phe	Ser	Phe	Asp	Leu	Thr	Gly	Asn	Ala	Lys	Phe	Gly	Gly
55																

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[illegible]

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	Ser	Lys	Asn	Gly	Asn	Thr	Glu	Asp	Tyr	Gly	Lys	Trp	Phe	Gly	Gln	Ala	
				420					425					430			
5	Ile	Ala	Gly	Asp	Asn	Ala	Val	Leu	Gly	Gly	Asp	Lys	Asp	Glu	Thr	Trp	
			435					440					445				
10	Ala	Lys	Phe	Asp	Leu	Thr	Ser	Thr	Ile	Asn	Arg	Asp	Arg	Asn	Ala	Pro	
		450					455					460					
15	Ser	Val	Ile	Met	Trp	Ser	Leu	Gly	Asn	Glu	Met	Met	Glu	Gly	Ile	Ser	
	465					470					475					480	
20	Gly	Ser	Val	Ser	Gly	Phe	Ser	Ala	Thr	Ser	Ala	Lys	Leu	Val	Ala	Trp	
					485					490					495		
25	Thr	Lys	Ala	Ala	Asp	Ser	Thr	Arg	Pro	Met	Thr	Tyr	Gly	Asp	Asn	Lys	
				500					505					510			
30	Ile	Lys	Ala	Asn	Trp	Asn	Glu	Ser	Asn	Thr	Met	Gly	Asp	Asn	Leu	Thr	
			515					520					525				
35	Ala	Asn	Gly	Gly	Val	Val	Gly	Thr	Asn	Tyr	Ser	Asp	Gly	Ala	Asn	Tyr	
		530					535					540					
40	Asp	Lys	Ile	Arg	Thr	Thr	His	Pro	Ser	Trp	Ala	Ile	Tyr	Gly	Ser	Glu	
	545					550					555					560	
45	Thr	Ala	Ser	Ala	Ile	Asn	Ser	Arg	Gly	Ile	Tyr	Asn	Arg	Thr	Thr	Gly	
					565					570					575		
50	Gly	Ala	Gln	Ser	Ser	Asp	Lys	Gln	Leu	Thr	Ser	Tyr	Asp	Asn	Ser	Ala	
				580					585					590			
55	Val	Gly	Trp	Gly	Ala	Val	Ala	Ser	Ser	Ala	Trp	Tyr	Asp	Val	Val	Gln	
			595					600					605				
60	Arg	Asp	Phe	Val	Ala	Gly	Thr	Tyr	Val	Trp	Thr	Gly	Phe	Asp	Tyr	Leu	
		610					615					620					
65	Gly	Glu	Pro	Thr	Pro	Trp	Asn	Gly	Thr	Gly	Ser	Gly	Ala	Val	Gly	Ser	
	625					630					635					640	
70	Trp	Pro	Ser	Pro	Lys	Asn	Ser	Tyr	Phe	Gly	Ile	Val	Asp	Thr	Ala	Gly	
					645					650					655		
75	Phe	Pro	Lys	Asp	Thr	Tyr	Tyr	Phe	Tyr	Gln	Ser	Gln	Trp	Asn	Asp	Asp	
				660					665					670			

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	Val	His	Thr	Leu	His	Ile	Leu	Pro	Ala	Trp	Asn	Glu	Asn	Val	Val	Ala	
				675				680					685				
5	Lys	Gly	Ser	Gly	Asn	Asn	Val	Pro	Val	Val	Val	Tyr	Thr	Asp	Ala	Ala	
		690					695					700					
10	Lys	Val	Lys	Leu	Tyr	Phe	Thr	Pro	Lys	Gly	Ser	Thr	Glu	Gln	Arg	Leu	
	705					710					715					720	
15	Ile	Gly	Glu	Lys	Ser	Phe	Thr	Lys	Lys	Thr	Thr	Ala	Ala	Gly	Tyr	Thr	
					725					730					735		
20	Tyr	Gln	Val	Tyr	Glu	Gly	Ser	Asp	Lys	Asp	Ser	Thr	Ala	His	Lys	Asn	
				740					745					750			
25	Met	Tyr	Leu	Thr	Trp	Asn	Val	Pro	Trp	Ala	Glu	Gly	Thr	Ile	Ser	Ala	
			755					760					765				
30	Glu	Ala	Tyr	Asp	Glu	Asn	Asn	Arg	Leu	Ile	Pro	Glu	Gly	Ser	Thr	Glu	
		770					775					780					
35	Gly	Asn	Ala	Ser	Val	Thr	Thr	Thr	Gly	Lys	Ala	Ala	Lys	Leu	Lys	Ala	
	785					790				795						800	
40	Asp	Ala	Asp	Arg	Lys	Thr	Ile	Thr	Ala	Asp	Gly	Lys	Asp	Leu	Ser	Tyr	
					805					810					815		
45	Ile	Glu	Val	Asp	Val	Thr	Asp	Ala	Asn	Gly	His	Ile	Val	Pro	Asp	Ala	
				820					825					830			
50	Ala	Asn	Arg	Val	Thr	Phe	Asp	Val	Lys	Gly	Ala	Gly	Lys	Leu	Val	Gly	
			835					840					845				
55	Val	Asp	Asn	Gly	Ser	Ser	Pro	Asp	His	Asp	Ser	Tyr	Gln	Ala	Asp	Asn	
		850					855					860					
60	Arg	Lys	Ala	Phe	Ser	Gly	Lys	Val	Leu	Ala	Ile	Val	Gln	Ser	Thr	Lys	
	865					870					875					880	
65	Glu	Ala	Gly	Glu	Ile	Thr	Val	Thr	Ala	Lys	Ala	Asp	Gly	Leu	Gln	Ser	
					885					890					895		
70	Ser	Thr	Val	Lys	Ile	Ala	Thr	Thr	Ala	Val	Pro	Gly	Thr	Ser	Thr	Glu	
				900					905					910			
75	Lys	Thr	Val	Arg	Ser	Phe	Tyr	Tyr	Ser	Arg	Asn	Tyr	Tyr	Val	Lys	Thr	
			915					920					925				

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	Gly	Asn	Lys	Pro	Ile	Leu	Pro	Ser	Asp	Val	Glu	Val	Arg	Tyr	Ser	Asp
	930						935					940				
5	Gly	Thr	Ser	Asp	Arg	Gln	Asn	Val	Thr	Trp	Asp	Ala	Val	Ser	Asp	Asp
	945					950					955					960
	Gln	Ile	Ala	Lys	Ala	Gly	Ser	Phe	Ser	Val	Ala	Gly	Thr	Val	Ala	Gly
10					965					970					975	
	Gln	Lys	Ile	Ser	Val	Arg	Val	Thr	Met	Ile	Asp	Glu	Ile	Gly	Ala	Leu
				980					985					990		
15	Leu	Asn	Tyr	Ser	Ala	Ser	Thr	Pro	Val	Gly	Thr	Pro	Ala	Val	Leu	Pro
			995					1000						1005		
	Gly	Ser	Arg	Pro	Ala	Val	Leu	Pro	Asp	Gly	Thr	Val	Thr	Ser	Ala	
20		1010					1015					1020				
	Asn	Phe	Ala	Val	His	Trp	Thr	Lys	Pro	Ala	Asp	Thr	Val	Tyr	Asn	
25		1025					1030					1035				
	Thr	Ala	Gly	Thr	Val	Lys	Val	Pro	Gly	Thr	Ala	Thr	Val	Phe	Gly	
		1040					1045					1050				
30	Lys	Glu	Phe	Lys	Val	Thr	Ala	Thr	Ile	Arg	Val	Gln	Arg	Ser	Gln	
		1055					1060					1065				
	Val	Thr	Ile	Gly	Ser	Ser	Val	Ser	Gly	Asn	Ala	Leu	Arg	Leu	Thr	
35		1070					1075					1080				
	Gln	Asn	Ile	Pro	Ala	Asp	Lys	Gln	Ser	Asp	Thr	Leu	Asp	Ala	Ile	
40		1085					1090					1095				
	Lys	Asp	Gly	Ser	Thr	Thr	Val	Asp	Ala	Asn	Thr	Gly	Gly	Gly	Ala	
		1100					1105					1110				
45	Asn	Pro	Ser	Ala	Trp	Thr	Asn	Trp	Ala	Tyr	Ser	Lys	Ala	Gly	His	
		1115					1120					1125				
	Asn	Thr	Ala	Glu	Ile	Thr	Phe	Glu	Tyr	Ala	Thr	Glu	Gln	Gln	Leu	
50		1130					1135					1140				
	Gly	Gln	Ile	Val	Met	Tyr	Phe	Phe	Arg	Asp	Ser	Asn	Ala	Val	Arg	
		1145					1150					1155				
55	Phe	Pro	Asp	Ala	Gly	Lys	Thr	Lys	Ile	Gln	Ile	Ser	Ala	Asp	Gly	

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	1160		1165		1170
5	Lys Asn Trp Thr Asp Leu Ala	Ala Thr Glu Thr Ile Ala Ala Gln			
	1175	1180	1185		
10	Glu Ser Ser Asp Arg Val Lys	Pro Tyr Thr Tyr Asp Phe Ala Pro			
	1190	1195	1200		
15	Val Gly Ala Thr Phe Val Arg	Val Thr Val Thr Asn Ala Asp Thr			
	1205	1210	1215		
20	Thr Thr Pro Ser Gly Val Val	Cys Ala Gly Leu Thr Glu Ile Glu			
	1220	1225	1230		
25	Leu Lys Thr Ala Thr Ser Lys	Phe Val Ala Asn Thr Ser Ala Ala			
	1235	1240	1245		
30	Leu Ser Ser Leu Thr Val Asn	Gly Thr Lys Val Ser Asp Ser Val			
	1250	1255	1260		
35	Leu Ala Ala Gly Ser Tyr Asn	Thr Pro Ala Ile Ile Ala Asp Val			
	1265	1270	1275		
40	Lys Ala Glu Gly Glu Gly Asn	Ala Ser Val Thr Val Leu Pro Ala			
	1280	1285	1290		
45	His Asp Asn Val Ile Arg Val	Ile Thr Glu Ser Glu Asp His Val			
	1295	1300	1305		
50	Thr Arg Lys Thr Phe Thr Ile	Asn Leu Gly Thr Glu Gln Glu Phe			
	1310	1315	1320		
55	Pro Ala Asp Ser Asp Glu Arg	Asp Gln His Gln His Gln His Gln			
	1325	1330	1335		
	His Gln Gln				
	1340				

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<211> 1752

<212> PRT

<213> Bifidobacterium bifidum

<400> 3

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Met Ala Val Arg Arg Leu Gly Gly Arg Ile Val Ala Phe Ala Ala Thr
1 5 10 15

5 Val Ala Leu Ser Ile Pro Leu Gly Leu Leu Thr Asn Ser Ala Trp Ala

10

15

20

25

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35

40

45

50

55

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	20	25	30
5	Val Glu Asp Ala Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr 35 40 45		
10	Pro Glu Val Val Tyr Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr 50 55 60		
15	Ser Asp Phe Asp Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln 65 70 75 80		
20	Ala Gln Asp Pro Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu 85 90 95		
25	Pro His Asp Tyr Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala 100 105 110		
30	Glu Ser Ala Tyr Leu Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe 115 120 125		
35	Thr Ile Asp Arg Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp 130 135 140		
40	Gly Val Tyr Met Asn Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly 145 150 155 160		
45	Thr His Pro Tyr Gly Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn 165 170 175		
50	Ala Lys Phe Gly Gly Glu Asn Thr Ile Val Val Lys Val Glu Asn Arg 180 185 190		
55	Leu Pro Ser Ser Arg Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val 195 200 205		
	Thr Leu Thr Val Thr Asp Gly Val His Val Gly Asn Asn Gly Val Ala 210 215 220		
	Ile Lys Thr Pro Ser Leu Ala Thr Gln Asn Gly Gly Asp Val Thr Met 225 230 235 240		
	Asn Leu Thr Thr Lys Val Ala Asn Asp Thr Glu Ala Ala Ala Asn Ile 245 250 255		
	Thr Leu Lys Gln Thr Val Phe Pro Lys Gly Gly Lys Thr Asp Ala Ala 260 265 270		

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	Ile	Gly	Thr	Val	Thr	Thr	Ala	Ser	Lys	Ser	Ile	Ala	Ala	Gly	Ala	Ser	
			275					280					285				
5	Ala	Asp	Val	Thr	Ser	Thr	Ile	Thr	Ala	Ala	Ser	Pro	Lys	Leu	Trp	Ser	
		290					295					300					
10	Ile	Lys	Asn	Pro	Asn	Leu	Tyr	Thr	Val	Arg	Thr	Glu	Val	Leu	Asn	Gly	
	305					310					315					320	
	Gly	Lys	Val	Leu	Asp	Thr	Tyr	Asp	Thr	Glu	Tyr	Gly	Phe	Arg	Trp	Thr	
					325					330					335		
15	Gly	Phe	Asp	Ala	Thr	Ser	Gly	Phe	Ser	Leu	Asn	Gly	Glu	Lys	Val	Lys	
				340					345					350			
20	Leu	Lys	Gly	Val	Ser	Met	His	His	Asp	Gln	Gly	Ser	Leu	Gly	Ala	Val	
			355					360					365				
	Ala	Asn	Arg	Arg	Ala	Ile	Glu	Arg	Gln	Val	Glu	Ile	Leu	Gln	Lys	Met	
		370					375					380					
25	Gly	Val	Asn	Ser	Ile	Arg	Thr	Thr	His	Asn	Pro	Ala	Ala	Lys	Ala	Leu	
	385					390					395					400	
30	Ile	Asp	Val	Cys	Asn	Glu	Lys	Gly	Val	Leu	Val	Val	Glu	Glu	Val	Phe	
					405					410					415		
	Asp	Met	Trp	Asn	Arg	Ser	Lys	Asn	Gly	Asn	Thr	Glu	Asp	Tyr	Gly	Lys	
35				420					425					430			
	Trp	Phe	Gly	Gln	Ala	Ile	Ala	Gly	Asp	Asn	Ala	Val	Leu	Gly	Gly	Asp	
			435					440					445				
40	Lys	Asp	Glu	Thr	Trp	Ala	Lys	Phe	Asp	Leu	Thr	Ser	Thr	Ile	Asn	Arg	
		450					455					460					
45	Asp	Arg	Asn	Ala	Pro	Ser	Val	Ile	Met	Trp	Ser	Leu	Gly	Asn	Glu	Met	
	465					470					475					480	
	Met	Glu	Gly	Ile	Ser	Gly	Ser	Val	Ser	Gly	Phe	Pro	Ala	Thr	Ser	Ala	
50				485						490					495		
	Lys	Leu	Val	Ala	Trp	Thr	Lys	Ala	Ala	Asp	Ser	Thr	Arg	Pro	Met	Thr	
				500					505					510			
55	Tyr	Gly	Asp	Asn	Lys	Ile	Lys	Ala	Asn	Trp	Asn	Glu	Ser	Asn	Thr	Met	
			515					520					525				

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	Gly	Asp	Asn	Leu	Thr	Ala	Asn	Gly	Gly	Val	Val	Gly	Thr	Asn	Tyr	Ser	
	530						535					540					
5	Asp	Gly	Ala	Asn	Tyr	Asp	Lys	Ile	Arg	Thr	Thr	His	Pro	Ser	Trp	Ala	
	545					550					555					560	
	Ile	Tyr	Gly	Ser	Glu	Thr	Ala	Ser	Ala	Ile	Asn	Ser	Arg	Gly	Ile	Tyr	
10					565					570					575		
	Asn	Arg	Thr	Thr	Gly	Gly	Ala	Gln	Ser	Ser	Asp	Lys	Gln	Leu	Thr	Ser	
				580					585					590			
15	Tyr	Asp	Asn	Ser	Ala	Val	Gly	Trp	Gly	Ala	Val	Ala	Ser	Ser	Ala	Trp	
			595					600					605				
	Tyr	Asp	Val	Val	Gln	Arg	Asp	Phe	Val	Ala	Gly	Thr	Tyr	Val	Trp	Thr	
20		610					615					620					
	Gly	Phe	Asp	Tyr	Leu	Gly	Glu	Pro	Thr	Pro	Trp	Asn	Gly	Thr	Gly	Ser	
25		625				630					635					640	
	Gly	Ala	Val	Gly	Ser	Trp	Pro	Ser	Pro	Lys	Asn	Ser	Tyr	Phe	Gly	Ile	
					645					650					655		
30	Val	Asp	Thr	Ala	Gly	Phe	Pro	Lys	Asp	Thr	Tyr	Tyr	Phe	Tyr	Gln	Ser	
				660					665					670			
	Gln	Trp	Asn	Asp	Asp	Val	His	Thr	Leu	His	Ile	Leu	Pro	Ala	Trp	Asn	
35			675					680					685				
	Glu	Asn	Val	Val	Ala	Lys	Gly	Ser	Gly	Asn	Asn	Val	Pro	Val	Val	Val	
		690					695					700					
40	Tyr	Thr	Asp	Ala	Ala	Lys	Val	Lys	Leu	Tyr	Phe	Thr	Pro	Lys	Gly	Ser	
	705					710					715					720	
	Thr	Glu	Lys	Arg	Leu	Ile	Gly	Glu	Lys	Ser	Phe	Thr	Lys	Lys	Thr	Thr	
45					725					730					735		
	Ala	Ala	Gly	Tyr	Thr	Tyr	Gln	Val	Tyr	Glu	Gly	Ser	Asp	Lys	Asp	Ser	
50				740					745					750			
	Thr	Ala	His	Lys	Asn	Met	Tyr	Leu	Thr	Trp	Asn	Val	Pro	Trp	Ala	Glu	
			755					760					765				
55	Gly	Thr	Ile	Ser	Ala	Glu	Ala	Tyr	Asp	Glu	Asn	Asn	Arg	Leu	Ile	Pro	
		770					775					780					

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	Glu	Gly	Ser	Thr	Glu	Gly	Asn	Ala	Ser	Val	Thr	Thr	Thr	Gly	Lys	Ala	785	790	795	800
5	Ala	Lys	Leu	Lys	Ala	Asp	Ala	Asp	Arg	Lys	Thr	Ile	Thr	Ala	Asp	Gly		805	810	815
10	Lys	Asp	Leu	Ser	Tyr	Ile	Glu	Val	Asp	Val	Thr	Asp	Ala	Asn	Gly	His		820	825	830
15	Ile	Val	Pro	Asp	Ala	Ala	Asn	Arg	Val	Thr	Phe	Asp	Val	Lys	Gly	Ala		835	840	845
20	Gly	Lys	Leu	Val	Gly	Val	Asp	Asn	Gly	Ser	Ser	Pro	Asp	His	Asp	Ser		850	855	860
25	Tyr	Gln	Ala	Asp	Asn	Arg	Lys	Ala	Phe	Ser	Gly	Lys	Val	Leu	Ala	Ile		865	870	875
30	Val	Gln	Ser	Thr	Lys	Glu	Ala	Gly	Glu	Ile	Thr	Val	Thr	Ala	Lys	Ala		885	890	895
35	Asp	Gly	Leu	Gln	Ser	Ser	Thr	Val	Lys	Ile	Ala	Thr	Thr	Ala	Val	Pro		900	905	910
40	Gly	Thr	Ser	Thr	Glu	Lys	Thr	Val	Arg	Ser	Phe	Tyr	Tyr	Ser	Arg	Asn		915	920	925
45	Tyr	Tyr	Val	Lys	Thr	Gly	Asn	Lys	Pro	Ile	Leu	Pro	Ser	Asp	Val	Glu		930	935	940
50	Val	Arg	Tyr	Ser	Asp	Gly	Thr	Ser	Asp	Arg	Gln	Asn	Val	Thr	Trp	Asp		945	950	955
55	Ala	Val	Ser	Asp	Asp	Gln	Ile	Ala	Lys	Ala	Gly	Ser	Phe	Ser	Val	Ala		965	970	975
	Gly	Thr	Val	Ala	Gly	Gln	Lys	Ile	Ser	Val	Arg	Val	Thr	Met	Ile	Asp		980	985	990
	Glu	Ile	Gly	Ala	Leu	Leu	Asn	Tyr	Ser	Ala	Ser	Thr	Pro	Val	Gly	Thr		995	1000	1005
	Pro	Ala	Val	Leu	Pro	Gly	Ser	Arg	Pro	Ala	Val	Leu	Pro	Asp	Gly			1010	1015	1020
	Thr	Val	Thr	Ser	Ala	Asn	Phe	Ala	Val	His	Trp	Thr	Lys	Pro	Ala					

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	1025					1030						1035			
5	Asp	Thr	Val	Tyr	Asn	Thr	Ala	Gly	Thr	Val	Lys	Val	Pro	Gly	Thr
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	Ala	Thr	Val	Phe	Gly	Lys	Glu	Phe	Lys	Val	Thr	Ala	Thr	Ile	Arg
10	1055						1060					1065			
	Val	Gln	Arg	Ser	Gln	Val	Thr	Ile	Gly	Ser	Ser	Val	Ser	Gly	Asn
	1070						1075					1080			
15	Ala	Leu	Arg	Leu	Thr	Gln	Asn	Ile	Pro	Ala	Asp	Lys	Gln	Ser	Asp
	1085						1090					1095			
	Thr	Leu	Asp	Ala	Ile	Lys	Asp	Gly	Ser	Thr	Thr	Val	Asp	Ala	Asn
20	1100						1105					1110			
	Thr	Gly	Gly	Gly	Ala	Asn	Pro	Ser	Ala	Trp	Thr	Asn	Trp	Ala	Tyr
	1115						1120					1125			
25	Ser	Lys	Ala	Gly	His	Asn	Thr	Ala	Glu	Ile	Thr	Phe	Glu	Tyr	Ala
	1130						1135					1140			
	Thr	Glu	Gln	Gln	Leu	Gly	Gln	Ile	Val	Met	Tyr	Phe	Phe	Arg	Asp
30	1145						1150					1155			
	Ser	Asn	Ala	Val	Arg	Phe	Pro	Asp	Ala	Gly	Lys	Thr	Lys	Ile	Gln
35	1160						1165					1170			
	Ile	Ser	Ala	Asp	Gly	Lys	Asn	Trp	Thr	Asp	Leu	Ala	Ala	Thr	Glu
	1175						1180					1185			
40	Thr	Ile	Ala	Ala	Gln	Glu	Ser	Ser	Asp	Arg	Val	Lys	Pro	Tyr	Thr
	1190						1195					1200			
	Tyr	Asp	Phe	Ala	Pro	Val	Gly	Ala	Thr	Phe	Val	Lys	Val	Thr	Val
45	1205						1210					1215			
	Thr	Asn	Ala	Asp	Thr	Thr	Thr	Pro	Ser	Gly	Val	Val	Cys	Ala	Gly
50	1220						1225					1230			
	Leu	Thr	Glu	Ile	Glu	Leu	Lys	Thr	Ala	Thr	Ser	Lys	Phe	Val	Thr
	1235						1240					1245			
55	Asn	Thr	Ser	Ala	Ala	Leu	Ser	Ser	Leu	Thr	Val	Asn	Gly	Thr	Lys
	1250						1255					1260			

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	Val	Ser	Asp	Ser	Val	Leu	Ala	Ala	Gly	Ser	Tyr	Asn	Thr	Pro	Ala
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5	Ile	Ile	Ala	Asp	Val	Lys	Ala	Glu	Gly	Glu	Gly	Asn	Ala	Ser	Val
	1280						1285					1290			
10	Thr	Val	Leu	Pro	Ala	His	Asp	Asn	Val	Ile	Arg	Val	Ile	Thr	Glu
	1295						1300					1305			
15	Ser	Glu	Asp	His	Val	Thr	Arg	Lys	Thr	Phe	Thr	Ile	Asn	Leu	Gly
	1310						1315					1320			
20	Thr	Glu	Gln	Glu	Phe	Pro	Ala	Asp	Ser	Asp	Glu	Arg	Asp	Tyr	Pro
	1325						1330					1335			
25	Ala	Ala	Asp	Met	Thr	Val	Thr	Val	Gly	Ser	Glu	Gln	Thr	Ser	Gly
	1340						1345					1350			
30	Thr	Ala	Thr	Glu	Gly	Pro	Lys	Lys	Phe	Ala	Val	Asp	Gly	Asn	Thr
	1355						1360					1365			
35	Ser	Thr	Tyr	Trp	His	Ser	Asn	Trp	Thr	Pro	Thr	Thr	Val	Asn	Asp
	1370						1375					1380			
40	Leu	Trp	Ile	Ala	Phe	Glu	Leu	Gln	Lys	Pro	Thr	Lys	Leu	Asp	Ala
	1385						1390					1395			
45	Leu	Arg	Tyr	Leu	Pro	Arg	Pro	Ala	Gly	Ser	Lys	Asn	Gly	Ser	Val
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<400> 4

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20

Val Glu Asp Ala Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr
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25

Ser Asp Phe Asp Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln
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30

Ala Gln Asp Pro Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu
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35

Pro His Asp Tyr Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala
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Thr Ile Asp Arg Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp
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45

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Ala Lys Phe Gly Gly Glu Asn Thr Ile Val Val Lys Val Glu Asn Arg
180 185 190

55

Leu Pro Ser Ser Arg Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val
195 200 205

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	Thr	Leu	Thr	Val	Thr	Asp	Gly	Val	His	Val	Gly	Asn	Asn	Gly	Val	Ala	
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5	Ile	Lys	Thr	Pro	Ser	Leu	Ala	Thr	Gln	Asn	Gly	Gly	Asn	Val	Thr	Met	
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10					245					250					255		
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				260					265					270			
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20		290					295					300					
	Ile	Lys	Asn	Pro	Asn	Leu	Tyr	Thr	Val	Arg	Thr	Glu	Val	Leu	Asn	Gly	
25	305					310					315					320	
	Gly	Lys	Val	Leu	Asp	Thr	Tyr	Asp	Thr	Glu	Tyr	Gly	Phe	Arg	Trp	Thr	
					325					330					335		
30	Gly	Phe	Asp	Ala	Thr	Ser	Gly	Phe	Ser	Leu	Asn	Gly	Glu	Lys	Val	Lys	
				340					345					350			
	Leu	Lys	Gly	Val	Ser	Met	His	His	Asp	Gln	Gly	Ser	Leu	Gly	Ala	Val	
35			355					360					365				
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		370					375					380					
40	Gly	Val	Asn	Ser	Ile	Arg	Thr	Thr	His	Asn	Pro	Ala	Ala	Lys	Ala	Leu	
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50				420					425					430			
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			435					440					445				
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	450		455		460											
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25	Gly	Asp 530	Asn	Leu	Thr	Ala	Asn 535	Gly	Gly	Val	Val	Gly 540	Thr	Asn	Tyr	Ser
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65	Val	Asp	Thr	Ala	Gly 660	Phe	Pro	Lys	Asp 665	Thr	Tyr	Tyr	Phe	Tyr 670	Gln	Ser
70	Gln	Trp	Asn	Asp	Asp	Val	His 680	Thr	Leu	His	Ile	Leu	Pro 685	Ala	Trp	Asn
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	Tyr	Thr	Asp	Ala	Ala	Lys	Val	Lys	Leu	Tyr	Phe	Thr	Pro	Lys	Gly	Ser	
	705					710					715					720	
5	Thr	Glu	Lys	Arg	Leu	Ile	Gly	Glu	Lys	Ser	Phe	Thr	Lys	Lys	Thr	Thr	
					725					730					735		
10	Ala	Ala	Gly	Tyr	Thr	Tyr	Gln	Val	Tyr	Glu	Gly	Ala	Asp	Lys	Asp	Ser	
				740					745					750			
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	770						775					780					
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				820					825					830			
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			835					840					845				
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50	Tyr	Gln	Ala	Asp	Asn	Arg	Lys	Ala	Phe	Ser	Gly	Lys	Val	Leu	Ala	Ile	
	865					870					875					880	
55	Val	Gln	Ser	Thr	Lys	Glu	Ala	Gly	Glu	Ile	Thr	Val	Thr	Ala	Lys	Ala	
					885					890					895		
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			915					920					925				
70	Tyr	Tyr	Val	Lys	Thr	Gly	Asn	Lys	Pro	Ile	Leu	Pro	Ser	Asp	Val	Glu	
	930						935					940					
75	Val	Arg	Tyr	Ser	Asp	Gly	Thr	Ser	Asp	Arg	Gln	Asn	Val	Thr	Trp	Asp	
	945					950					955					960	

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	Ala	Val	Ser	Asp	Asp	Gln	Ile	Ala	Lys	Ala	Gly	Ser	Phe	Ser	Val	Ala	
					965					970					975		
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				980					985					990			
	Glu	Ile	Gly	Ala	Leu	Leu	Asn	Tyr	Ser	Ala	Ser	Thr	Pro	Val	Gly	Thr	
10			995				1000						1005				
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		1010					1015					1020					
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		1025					1030					1035					
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20		1040					1045					1050					
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		1115					1120					1125					
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		1130					1135					1140					
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50		1160					1165					1170					
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	Tyr	Asp	Phe	Ala	Pro	Val	Gly	Ala	Thr	Phe	Val	Lys	Val	Thr	Val
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	1235						1240					1245			
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	1250						1255					1260			
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	1265						1270					1275			
25	Ile	Ile	Ala	Asp	Val	Lys	Ala	Glu	Gly	Glu	Gly	Asn	Ala	Ser	Val
	1280						1285					1290			
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	1295						1300					1305			
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	1310						1315					1320			
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	1325						1330					1335			
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	1340						1345					1350			
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	1355						1360					1365			
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	1370						1375					1380			
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	1385						1390					1395			
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	1400						1405					1410			
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	1550						1555					1560			
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	1565						1570					1575			
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	1580						1585					1590			
55	Gly	Asp	Ala	Phe	Asp	Pro	Ala	Gly	Leu	Val	Leu	Gln	Leu	Asn	Tyr
	1595						1600					1605			
60	Asp	Asp	Asp	Ser	Thr	Gly	Thr	Val	Thr	Trp	Asn	Thr	Gln	Thr	Ala
	1610						1615					1620			
65	Gly	Asp	Phe	Thr	Phe	Lys	Pro	Ala	Leu	Asp	Ala	Lys	Leu	Lys	Val
	1625						1630					1635			
70	Thr	Asp	Lys	Thr	Val	Thr	Val	Thr	Tyr	Gln	Gly	Lys	Ser	Ala	Val
	1640						1645					1650			
75	Ile	Asp	Ile	Thr	Val	Ser	Gln	Pro	Ala	Pro	Thr	Val	Ser	Lys	Thr
	1655						1660					1665			

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	Asp	Leu	Asp	Lys	Ala	Ile	Lys	Ala	Ile	Glu	Ala	Lys	Asn	Pro	Asp
	1670						1675					1680			
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	1685						1690					1695			
10	Met	Ala	His	Ala	Lys	Ala	Val	Ile	Ala	Asp	Asp	Ser	Ala	Thr	Gln
	1700						1705					1710			
15	Gln	Asp	Val	Asp	Lys	Ala	Leu	Lys	Ala	Leu	Thr	Asp	Ala	Tyr	Ala
	1715						1720					1725			
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	1730						1735					1740			
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	1745						1750					1755			
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	1760						1765					1770			
35	Leu	Ala	His	Ala	Lys	Ala	Val	Ile	Ala	Ser	Asp	Ser	Ala	Thr	Gln
	1775						1780					1785			
40	Gln	Asp	Val	Asp	Ala	Ala	Leu	Gly	Ala	Leu	Thr	Ser	Ala	Arg	Asp
	1790						1795					1800			
45	Gly	Leu	Thr	Glu	Lys	Gly	Glu	Val	Lys	Pro	Asp	Pro	Lys	Pro	Glu
	1805						1810					1815			
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	1835						1840					1845			
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	1865						1870					1875			
70	Met	Leu	Asn	Asp	Ala	Arg	Ala	Ala	Leu	Lys	Glu	Lys	Pro	Gly	Arg
	1880						1885					1890			
75	Ile	Ile	Ala	Ile	Ile	Asp	Gly	Gly	Ala	Leu	Ser	Lys	Thr	Gly	Ala
	1895						1900					1905			

Ser Val Ala Ile Ile Ala Ser Val Ala Ala Ala Met Lys Ala Val
 1910 1915 1920

Gly Ala Gly Val Met Ala Leu Arg Pro Pro Lys Trp
 1925 1930 1935

Claims

1. A process for producing a fermented milk product comprising the steps of
 - 1) adding a starter culture comprising at least one lactic acid bacteria strain to a milk base,
 - 2) fermenting the milk for a period of time until a target pH is reached,
 - 3) wherein the starter culture comprises at least one lactose-deficient strain, which is capable of metabolizing a non-lactose carbohydrate, and
 - 4) adding a low pH stable lactase to the process either at the start, during or at the end of the fermentation step, wherein the low pH stable lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.
2. A process according to claim 1, wherein the low pH stable lactase retains its activity at a temperature of 10 °C and a pH of 6.0 at a level of at least 10 % as compared to its activity at the optimum temperature of the lactase.
3. A process according to claim 1 or 2, wherein the lactose-deficient strain is capable of metabolizing a non-lactose carbohydrate selected from the group consisting of sucrose, galactose and glucose.
4. A process according to any of the preceding claims, wherein non-lactose carbohydrate is added to the milk base at the start of the fermentation step.
5. A process according to claim 4, wherein the non-lactose carbohydrate is added to the milk base in an amount measured so as to become depleted and hence result in stopping the growth of lactic acid bacteria and in stopping the fermentation.
6. A process according to any of the preceding claims, wherein the low pH stable lactase is added to the milk base at the start of the fermentation step.
7. A process according to claim 6, wherein no non-lactose carbohydrate is added to the fermentation step, and wherein at least one lactose-deficient lactic acid strain of the starter culture is capable of metabolizing a carbohydrate selected from the group consisting of glucose and galactose.
8. A process according to any of claims 1-5, wherein the low pH stable lactase is added to the milk base at the end of the fermentation step.
9. A process according to any of claims 1-8, wherein the lactose-deficient strain is selected from the group consisting of lactose-deficient *Streptococcus thermophilus* and lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus*.
10. A process according to claim 9, wherein the lactose-deficient strain is selected from the group consisting of:
 - (a) a *Streptococcus thermophilus* strain, which strain is:
 - (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28952;
 - (ii) or a strain derived from DSM 28952, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal;
 - (b) a *Streptococcus thermophilus* strain, which strain is:
 - (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH,

Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28953;
(ii) or a strain derived from DSM 28953, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal;

(c) a *Lactobacillus delbrueckii* ssp. *bulgaricus* strain, which strain is:

- (i) the strain deposited with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, on 2014-06-12 under the accession no. DSM 28910;
- (ii) or a strain derived from DSM 28910, wherein the derived strain is further characterized as having the ability to generate white colonies on a medium containing lactose and X-Gal.

11. A process according to claim 9 or 10, wherein the starter culture contains both at least one lactose-deficient *Streptococcus thermophilus* and at least one lactose-deficient *Lactobacillus delbrueckii* subsp. *bulgaricus*.

12. A process according to any of claims 9-11, wherein the starter culture contains both at least one *Streptococcus thermophilus* and at least one *Lactobacillus delbrueckii* subsp. *bulgaricus*, and wherein all *Streptococcus thermophilus* and all *Lactobacillus delbrueckii* subsp. *bulgaricus* strains are lactose-deficient.

13. Use in a process for producing a fermented milk product comprising the steps of

- 1) adding a starter culture comprising at least one lactic acid bacteria strain to a milk base,
- 2) fermenting the milk for a period of time until a target pH is reached,
- of
- 3) the starter culture comprising at least one lactose-deficient strain, which is capable of metabolizing a non-lactose carbohydrate, and
- 4) a low pH stable lactase added to the process either at the start, during or at the end of the fermentation step, wherein the low pH stable lactase retains its activity at a pH of 5.0 and a temperature of 37 °C at a level of at least 5 % as compared to its activity at the optimum pH of the lactase.

14. Use according to claim 13 to increase the texture of the fermented milk product as compared to using lactose-deficient lactic acid bacteria and no lactase and as compared to using a low pH stable lactase and lactose-positive lactic acid bacteria.

Patentansprüche

1. Prozess zum Produzieren eines fermentierten Milchprodukts, umfassend die folgenden Schritte:

- 1) Hinzufügen einer Starterkultur, die zumindest einen Milchsäurebakterienstamm umfasst, zu einer Milchbasis,
- 2) Fermentierenlassen der Milch für einen Zeitraum, bis ein Ziel-pH-Wert erreicht ist,
- 3) wobei die Starterkultur zumindest einen laktosedefizienten Stamm umfasst, der in der Lage ist, ein Nicht-Laktose-Kohlenhydrat zu metabolisieren, und
- 4) Hinzufügen einer stabilen Laktase mit niedrigem pH-Wert zu dem Prozess, entweder zu Beginn, während oder gegen Ende des Fermentierungsschrittes, wobei die stabile Laktase mit niedrigem pH-Wert ihre Aktivität verglichen mit ihrer Aktivität bei dem optimalen pH-Wert der Laktase bei einem pH-Wert von 5,0 und einer Temperatur von 37 °C auf einem Niveau von zumindest 5 % hält.

2. Prozess nach Anspruch 1, wobei die stabile Laktase mit niedrigem pH-Wert ihre Aktivität verglichen mit ihrer Aktivität bei der optimalen Temperatur der Laktase bei einer Temperatur von 10 °C und einem pH-Wert von 6,0 auf einem Niveau von zumindest 10 % hält.

3. Prozess nach Anspruch 1 oder 2, wobei der laktosedefiziente Stamm in der Lage ist, ein Nicht-Laktose-Kohlenhydrat zu metabolisieren, das aus der Gruppe bestehend aus Saccharose, Galactose und Glucose ausgewählt ist.

4. Prozess nach einem der vorangehenden Ansprüche, wobei das Nicht-Laktose-Kohlenhydrat zu Beginn des Fermentierungsschrittes zu der Milchbasis hinzugefügt wird.

5. Prozess nach Anspruch 4, wobei das Nicht-Laktose-Kohlenhydrat in einer Menge, die abgemessen wird, um gesättigt

zu werden und somit zu einem Anhalten des Wachstums der Milchsäurebakterien und einem Anhalten der Fermentierung zu führen, hinzugefügt wird.

6. Prozess nach einem der vorangehenden Ansprüche, wobei die stabile Laktase mit niedrigem pH-Wert zu Beginn des Fermentierungsschrittes zu der Milchbasis hinzugefügt wird.

7. Prozess nach Anspruch 6, wobei kein Nicht-Laktose-Kohlenhydrat zu dem Fermentierungsschritt hinzugefügt wird und wobei zumindest ein laktosedefizienter Milchsäurestamm der Starterkultur in der Lage ist, ein Kohlenhydrat zu metabolisieren, das aus der Gruppe bestehend aus Glucose und Galactose ausgewählt ist.

8. Prozess nach einem der Ansprüche 1-5, wobei die stabile Laktase mit niedrigem pH-Wert gegen Ende des Fermentierungsschrittes zu der Milchbasis hinzugefügt wird.

9. Prozess nach einem der Ansprüche 1-8, wobei der laktosedefiziente Stamm aus der Gruppe bestehend aus laktosedefizientem *Streptococcus thermophilus* und laktosedefizientem *Lactobacillus delbrueckii subsp. bulgaricus* ausgewählt ist.

10. Prozess nach Anspruch 9, wobei der laktosedefiziente Stamm aus der Gruppe ausgewählt ist, die aus Folgendem besteht:

(a) einem *Streptococcus-thermophilus*-Stamm, wobei es sich bei dem Stamm um Folgendes handelt:

(i) den Stamm, der am 12.06.2014 bei der DSMZ-Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, mit der Hinterlegungsnummer DSM 28952 hinterlegt wurde;

(ii) oder einen Stamm, der von DSM 28952 abgeleitet wurde, wobei der abgeleitete Stamm ferner **dadurch gekennzeichnet ist, dass** er die Fähigkeit aufweist, weiße Kolonien auf einem Medium zu erzeugen, das Lactose und X-Gal enthält;

(b) einem *Streptococcus-thermophilus*-Stamm, wobei es sich bei dem Stamm um Folgendes handelt:

(i) den Stamm, der am 12.06.2014 bei der DSMZ-Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, mit der Hinterlegungsnummer DSM 28953 hinterlegt wurde;

(ii) oder einen Stamm, der von DSM 28953 abgeleitet wurde, wobei der abgeleitete Stamm ferner **dadurch gekennzeichnet ist, dass** er die Fähigkeit aufweist, weiße Kolonien auf einem Medium zu erzeugen, das Lactose und X-Gal enthält;

(c) einen *Lactobacillus-delbrueckii-ssp.-bulgaricus*-Stamm, wobei es sich bei dem Stamm um Folgendes handelt:

(i) den Stamm, der am 12.06.2014 bei der DSMZ-Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, mit der Hinterlegungsnummer DSM 28910 hinterlegt wurde;

(ii) oder einen Stamm, der von DSM 28910 abgeleitet wurde, wobei der abgeleitete Stamm ferner **dadurch gekennzeichnet ist, dass** er die Fähigkeit aufweist, weiße Kolonien auf einem Medium zu erzeugen, das Lactose und X-Gal enthält.

11. Prozess nach Anspruch 9 oder 10, wobei die Starterkultur sowohl zumindest einen laktosedefizienten *Streptococcus thermophilus* als auch zumindest einen laktosedefizienten *Lactobacillus delbrueckii subsp. bulgaricus* enthält.

12. Prozess nach einem der Ansprüche 9-11, wobei die Starterkultur sowohl zumindest einen *Streptococcus thermophilus* und zumindest einen *Lactobacillus delbrueckii subsp. bulgaricus* enthält und wobei alle *Streptococcus-thermophilus*- und alle *Lactobacillus-delbrueckii-subsp.-bulgaricus*-Stämme laktosedefizient sind.

13. Verwendung in einem Prozess zum Produzieren eines fermentierten Milchprodukts, umfassend die folgenden Schritte:

- 1) Hinzufügen einer Starterkultur, die zumindest einen Milchsäurebakterienstamm umfasst, zu einer Milchbasis,
- 2) Fermentierenlassen der Milch für einen Zeitraum, bis ein Ziel-pH-Wert
- 3) der Starterkultur erreicht ist, die zumindest einen laktosedefizienten Stamm umfasst, der in der Lage ist, ein
- 4) wobei eine stabile Laktase mit niedrigem pH-Wert entweder zu Beginn, während oder gegen Ende des
- Fermentierungsschrittes zu dem Prozess hinzugefügt wird, wobei die stabile Laktase mit niedrigem pH-Wert
- ihre Aktivität verglichen mit ihrer Aktivität bei dem optimalen pH-Wert der Laktase bei einem pH-Wert von 5,0
- und einer Temperatur von 37 °C auf einem Niveau von zumindest 5 % hält.

14. Verwendung nach Anspruch 13, um die Textur des fermentierten Milchprodukts verglichen mit Verwenden von laktosedefizienten Milchsäurebakterien und keiner Lactase und verglichen mit Verwendung einer stabilen Lactase mit niedrigem pH-Wert und laktosepositiven Milchsäurebakterien zu erhöhen.

Revendications

1. Procédé de production d'un produit laitier fermenté comprenant les étapes

- 1) d'ajout d'une culture starter comprenant au moins une souche de bactéries lactiques à une base de lait,
- 2) de fermentation du lait pendant un certain temps jusqu'à ce qu'un pH cible soit atteint,
- 3) dans lequel la culture starter comprend au moins une souche déficiente en lactose, qui est capable de métaboliser un glucide sans lactose, et
- 4) d'ajout d'une lactase stable à pH bas au procédé, au début, pendant ou à la fin de l'étape de fermentation, dans lequel la lactase stable à pH bas maintient son activité à un pH de 5,0 et une température de 37 °C à un niveau d'au moins 5 % par rapport à son activité au pH optimal de la lactase.

2. Procédé selon la revendication 1, dans lequel la lactase stable à pH bas maintient son activité à une température de 10 °C et un pH de 6,0 à un niveau d'au moins 10 % par rapport à son activité à la température optimale de la lactase.

3. Procédé selon la revendication 1 ou 2, dans lequel la souche déficiente en lactose est capable de métaboliser un glucide sans lactose sélectionné parmi le groupe constitué de saccharose, de galactose et de glucose.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel un glucide sans lactose est ajouté à la base de lait au début de l'étape de fermentation.

5. Procédé selon la revendication 4, dans lequel le glucide sans lactose est ajouté à la base de lait en une quantité mesurée de manière à s'épuiser et donc entraîner l'arrêt de la croissance des bactéries lactiques et l'arrêt de la fermentation.

6. Procédé selon l'une quelconque des revendications précédentes, dans lequel la lactase stable à pH bas est ajoutée à la base de lait au début de l'étape de fermentation.

7. Procédé selon la revendication 6, dans lequel aucun glucide sans lactose n'est ajouté à l'étape de fermentation, et dans lequel au moins une souche d'acide lactique déficiente en lactose de la culture starter est capable de métaboliser un glucide sélectionné parmi le groupe constitué de glucose et de galactose.

8. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel la lactase stable à pH bas est ajoutée à la base de lait à la fin de l'étape de fermentation.

9. Procédé selon l'une quelconque des revendications 1 à 8, dans lequel la souche déficiente en lactose est sélectionnée parmi le groupe constitué de *Streptococcus thermophilus* déficient en lactose et de *Lactobacillus delbrueckii* sous-espèce *bulgaricus* déficient en lactose.

10. Procédé selon la revendication 9, dans lequel la souche déficiente en lactose est sélectionnée parmi le groupe constitué :

- (a) d'une souche de *Streptococcus thermophilus*, laquelle souche est :

- (i) la souche déposée auprès de DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, le 12-06-2014 sous le n° d'entrée DSM 28952 ;
- (ii) ou une souche dérivée de DSM 28952, dans lequel la souche dérivée est en outre **caractérisée** comme ayant la capacité à générer des colonies blanches sur un milieu contenant du lactose et X-Gal ;

(b) d'une souche de *Streptococcus thermophilus*, laquelle souche est :

- (i) la souche déposée auprès de DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, le 12-06-2014 sous le n° d'entrée DSM 28953 ;
- (ii) ou une souche dérivée de DSM 28953, dans lequel la souche dérivée est en outre **caractérisée** comme ayant la capacité à générer des colonies blanches sur un milieu contenant du lactose et X-Gal ;

(c) d'une souche de *Streptococcus delbrueckii* sous-espèce *bulgaricus*, laquelle souche est :

- (i) la souche déposée auprès de DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, le 12-06-2014 sous le n° d'entrée DSM 28910 ;
- (ii) ou une souche dérivée de DSM 28910, dans lequel la souche dérivée est en outre **caractérisée** comme ayant la capacité à générer des colonies blanches sur un milieu contenant du lactose et X-Gal.

11. Procédé selon la revendication 9 ou 10, dans lequel la culture starter contient à la fois au moins un *Streptococcus thermophilus* déficient en lactose et au moins un *Lactobacillus delbrueckii* sous-espèce *bulgaricus* déficient en lactose.

12. Procédé selon l'une quelconque des revendications 9 à 11, dans lequel la culture starter contient à la fois au moins un *Streptococcus thermophilus* et au moins un *Lactobacillus delbrueckii* sous-espèce *bulgaricus*, et dans lequel toutes les souches de *Streptococcus thermophilus* et toutes les souches de *Lactobacillus delbrueckii* sous-espèce *bulgaricus* sont déficientes en lactose.

13. Utilisation dans un procédé de production d'un produit laitier fermenté comprenant les étapes

- 1) d'ajout d'une culture starter comprenant au moins une souche de bactéries lactiques à une base de lait,
- 2) de fermentation du lait pendant un certain temps jusqu'à ce qu'un pH cible soit atteint,
- 3) la culture starter comprenant au moins une souche déficiente en lactose, qui est capable de métaboliser un glucide sans lactose, et
- 4) une lactase stable à pH bas ajoutée au procédé, au début, pendant ou à la fin de l'étape de fermentation, dans laquelle la lactase stable à pH bas maintient son activité à un pH de 5,0 et une température de 37 °C à un niveau d'au moins 5 % par rapport à son activité au pH optimal de la lactase.

14. Utilisation selon la revendication 13 pour augmenter la texture du produit laitier fermenté par rapport à l'utilisation de bactéries lactiques déficientes en lactose et sans lactase et par rapport à l'utilisation d'une lactase stable à pH bas et de bactéries lactiques positives pour le lactose.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2009071539 A [0002] [0023]
- WO 2013160413 A [0003]
- EP 2957180 A1 [0004]

Non-patent literature cited in the description

- **NEEDLEMAN ; WUNSCH.** *J. Mol. Biol.*, 1970, vol. 48, 443-453 [0035]
- **RICE et al.** EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genetics*, 2000, vol. 16, 276-277 [0035]
- **WERNER, W. et al.** *Z. analyt. Chem.*, 1970, vol. 252, 224 [0112] [0126] [0133]
- **T K GOULAS.** *Appl Microbiol Biotechnol*, 2007, vol. 76, 1365-1372 [0215]
- **T GOULAS.** *Appl Microbiol Biotechnol*, 2009, vol. 82, 1079-1088 [0215]
- **T GOULAS.** *Appl Microbiol Biotechnol*, 2009, vol. 84, 899-907 [0215]